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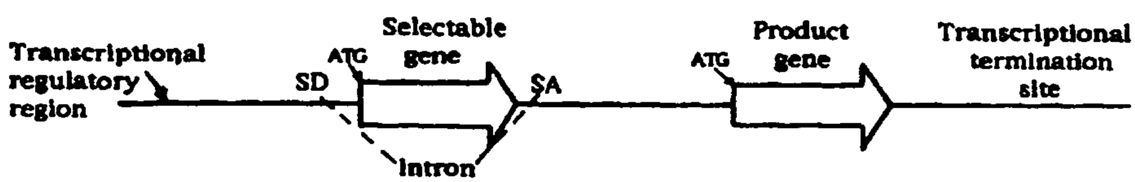

(57) Abstract

A method for selecting recombinant host cells expressing high levels of a desired protein is described. This method utilizes eukaryotic host cells harboring a DNA construct comprising a selectable gene (preferably an amplifiable gene) and a product gene provided 3' to the selectable gene. The selectable gene is positioned within an intron defined by a splice donor site and a splice acceptor site and the selectable gene and product gene are under the transcriptional control of a single transcriptional regulatory region. The splice donor site is generally an efficient splice donor site and thereby regulates expression of the product gene using the transcriptional regulatory region. The transfected cells are cultured so as to express the gene encoding the product in a selective medium comprising an amplifying agent for sufficient time to allow amplification to occur, whereupon either the desired product is recovered or cells having multiple copies of the product gene are identified.

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METHOD FOR SELECTING HIGH-EXPRESSING HOST CELLS

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates to a method of selecting for high-expressing host cells, a method of producing a protein of interest in high yields and a method of producing eukaryotic cells having multiple copies of a sequence encoding a protein of interest.

Description of Background and Related Art

The discovery of methods for introducing DNA into living host cells in a functional form has provided the key to understanding many fundamental biological processes, and has made possible the production of important proteins and other molecules in commercially useful quantities.

Despite the general success of such gene transfer methods, several common problems exist that may limit the efficiency with which a gene encoding a desired protein can be introduced into and expressed in a host cell. One problem is knowing when the gene has been successfully transferred into recipient cells. A second problem is distinguishing between those cells that contain the gene and those that have survived the transfer procedures but do not contain the gene. A third problem is identifying and isolating those cells that contain the gene and that are expressing high levels of the protein encoded by the gene.

In general, the known methods for introducing genes into eukaryotic cells tend to be highly inefficient. Of the cells in a given culture, only a small proportion take up and express exogenously added DNA, and an even smaller proportion stably maintain that DNA.

Identification of those cells that have incorporated a product gene encoding a desired protein typically is achieved by introducing into the same cells another gene, commonly referred to as a selectable gene, that encodes a selectable marker. A selectable marker is a protein that is necessary for the growth or survival of a host cell under the particular culture conditions chosen, such as an enzyme that confers resistance to an antibiotic or other drug, or an enzyme that compensates for a metabolic or catabolic defect in the host cell. For example, selectable genes commonly used with eukaryotic cells include the genes for aminoglycoside phosphotransferase (APH), hygromycin phosphotransferase (hyg), dihydrofolate reductase (DHFR), thymidine kinase (tk), neomycin, puromycin, glutamine synthetase, and asparagine synthetase.

The method of identifying a host cell that has incorporated one gene on the basis of expression by the host cell of a second incorporated gene encoding a selectable marker is referred to as cotransfectation (or cotransfection). In that method, a gene encoding a desired polypeptide and a selection gene typically are introduced into the host cell simultaneously, although they may be introduced sequentially. In the case of simultaneous cotransfectation, the gene encoding the desired polypeptide

and the selectable gene may be present on a single DNA molecule or on separate DNA molecules prior to being introduced into the host cells. Wigler *et al.*, Cell, 16:777 (1979). Cells that have incorporated the gene encoding the desired polypeptide then are identified or isolated by culturing the cells under conditions that preferentially allow for the growth or survival of those cells that synthesize the selectable marker encoded by the selectable gene.

The level of expression of a gene introduced into a eukaryotic host cell depends on multiple factors, including gene copy number, efficiency of transcription, messenger RNA (mRNA) processing, stability, and translation efficiency. Accordingly, high level expression of a desired polypeptide typically will involve optimizing one or more of those factors.

For example, the level of protein production may be increased by covalently joining the coding sequence of the gene to a "strong" promoter or enhancer that will give high levels of transcription. Promoters and enhancers are nucleotide sequences that interact specifically with proteins in a host cell that are involved in transcription. Kriegler, Meth. Enzymol., 185:512 (1990); Maniatis *et al.*, Science, 236:1237 (1987). Promoters are located upstream of the coding sequence of a gene and facilitate transcription of the gene by RNA polymerase. Among the eukaryotic promoters that have been identified as strong promoters for high-level expression are the SV40 early promoter, adenovirus major late promoter, mouse metallothionein-I promoter, Rous sarcoma virus long terminal repeat, and human cytomegalovirus immediate early promoter (CMV).

Enhancers stimulate transcription from a linked promoter. Unlike promoters, enhancers are active when placed downstream from the transcription initiation site or at considerable distances from the promoter, although in practice enhancers may overlap physically and functionally with promoters. For example, all of the strong promoters listed above also contain strong enhancers. Bendig, Genetic Engineering, 7:91 (Academic Press, 1988).

The level of protein production also may be increased by increasing the gene copy number in the host cell. One method for obtaining high gene copy number is to directly introduce into the host cell multiple copies of the gene, for example, by using a large molar excess of the product gene relative to the selectable gene during cotransfectation. Kaufman, Meth. Enzymol., 185:537 (1990). With this method, however, only a small proportion of the cotransfected cells will contain the product gene at high copy number. Furthermore, because no generally applicable, convenient method exists for distinguishing such cells from the majority of cells that contain fewer copies of the product gene, laborious and time-consuming screening methods typically are required to identify the desired high-copy number transfectants.

Another method for obtaining high gene copy number involves cloning the gene in a vector that is capable of replicating autonomously in the host cell. Examples of such vectors include mammalian expression vectors

derived from Epstein-Barr virus or bovine papilloma virus, and yeast 2-micron plasmid vectors. Stephens & Hentschel, Biochem. J., 248:1 (1987); Yates *et al.*, Nature, 313:812 (1985); Beggs, Genetic Engineering, 2:175 (Academic Press, 1981).

Yet another method for obtaining high gene copy number involves gene amplification in the host cell. Gene amplification occurs naturally in eukaryotic cells at a relatively low frequency. Schimke, J. Biol. Chem., 263:5989 (1988). However, gene amplification also may be induced, or at least selected for, by exposing host cells to appropriate selective pressure. For example, in many cases it is possible to introduce a product gene together with an amplifiable gene into a host cell and subsequently select for amplification of the marker gene by exposing the cotransfected cells to sequentially increasing concentrations of a selective agent. Typically the product gene will be coamplified with the marker gene under such conditions.

The most widely used amplifiable gene for that purpose is a DHFR gene, which encodes a dihydrofolate reductase enzyme. The selection agent used in conjunction with a DHFR gene is methotrexate (Mtx). A host cell is cotransfected with a product gene encoding a desired protein and a DHFR gene, and transfectants are identified by first culturing the cells in culture medium that contains Mtx. A suitable host cell when a wild-type DHFR gene is used is the Chinese Hamster Ovary (CHO) cell line deficient in DHFR activity, prepared and propagated as described by Urlaub & Chasin, Proc. Nat. Acad. Sci. USA, 77:4216 (1980). The transfected cells then are exposed to successively higher amounts of Mtx. This leads to the synthesis of multiple copies of the DHFR gene, and concomitantly, multiple copies of the product gene. Schimke, J. Biol. Chem., 263:5989 (1988); Axel *et al.*, U.S. Patent No. 4,399,216; Axel *et al.*, U.S. Patent No. 4,634,665. Other references directed to co-transfection of a gene together with a genetic marker that allows for selection and subsequent amplification include Kaufman in Genetic Engineering, ed. J. Setlow (Plenum Press, New York), Vol. 9 (1987); Kaufman and Sharp, J. Mol. Biol., 159:601 (1982); Ringold *et al.*, J. Mol. Appl. Genet., 1:165-175 (1981); Kaufman *et al.*, Mol. Cell Biol., 5:1750-1759 (1985); Kaetzel and Nilson, J. Biol. Chem., 263:6244-6251 (1988); Hung *et al.*, Proc. Natl. Acad. Sci. USA, 83:261-264 (1986); Kaufman *et al.*, EMBO J., 6:87-93 (1987); Johnston and Kucey, Science, 242:1551-1554 (1988); Urlaub *et al.*, Cell, 33:405-412 (1983).

To extend the DHFR amplification method to other cell types, a mutant DHFR gene that encodes a protein with reduced sensitivity to methotrexate may be used in conjunction with host cells that contain normal numbers of an endogenous wild-type DHFR gene. Simonsen and Levinson, Proc. Natl. Acad. Sci. USA, 80:2495 (1983); Wigler *et al.*, Proc. Natl. Acad. Sci. USA, 77:3567-3570 (1980); Haber and Schimke, Somatic Cell Genetics, 8:499-508 (1982).

Alternatively, host cells may be co-transfected with the product gene, a DHFR gene, and a dominant selectable gene, such as a neo^r gene. Kim

and Wold, Cell, 42:129 (1985); Capon *et al.*, U.S. Pat. No. 4,965,199. Transfectants are identified by first culturing the cells in culture medium containing neomycin (or the related drug G418), and the transfectants so identified then are selected for amplification of the DHFR gene and the product gene by exposure to successively increasing amounts of Mtx.

As will be appreciated from this discussion, the selection of recombinant host cells that express high levels of a desired protein generally is a multi-step process. In the first step, initial transfectants are selected that have incorporated the product gene and the selectable gene. In subsequent steps, the initial transfectants are subject to further selection for high-level expression of the selectable gene and then random screening for high-level expression of the product gene. To identify cells expressing high levels of the desired protein, typically one must screen large numbers of transfectants. The majority of transfectants produce less than maximal levels of the desired protein. Further, Mtx resistance in DHFR transformants is at least partially conferred by varying degrees of gene amplification. Schimke, Cell, 37:705-713 (1984). The inadequacies of co-expression of the non-selected gene have been reported by Wold *et al.*, Proc. Natl. Acad. Sci. USA, 76:5684-5688 (1979). Instability of the amplified DNA is reported by Kaufman and Schimke, Mol. Cell Biol., 1:1069-1076 (1981); Haber and Schimke, Cell, 26:355-362 (1981); and Fedespiel *et al.*, J. Biol. Chem., 259:9127-9140 (1984).

Several methods have been described for directly selecting such recombinant host cells in a single step. One strategy involves co-transfecting host cells with a product gene and a DHFR gene, and selecting those cells that express high levels of DHFR by directly culturing in medium containing a high concentration of Mtx. Many of the cells selected in that manner also express the co-transfected product gene at high levels. Page and Sydenham, Bio/Technology, 9:64 (1991). This method for single-step selection suffers from certain drawbacks that limit its usefulness. High-expressing cells obtained by direct culturing in medium containing a high level of a selection agent may have poor growth and stability characteristics, thus limiting their usefulness for long-term production processes. Page and Snyderman, Bio/Technology, 9:64 (1991). Single-step selection for high-level resistance to Mtx may produce cells with an altered, Mtx-resistant DHFR enzyme, or cells that have altered Mtx transport properties, rather than cells containing amplified genes. Haber *et al.*, J. Biol. Chem., 256:9501 (1981); Assaraf and Schimke, Proc. Natl. Acad. Sci. USA, 84:7154 (1987).

Another method involves the use of polycistronic mRNA expression vectors containing a product gene at the 5′ end of the transcribed region and a selectable gene at the 3′ end. Because translation of the selectable gene at the 3′ end of the polycistronic mRNA is inefficient, such vectors exhibit preferential translation of the product gene and require high levels of polycistronic mRNA to survive selection. Kaufman, Meth.

Enzymol., 185:487 (1990); Kaufman, Meth. Enzymol., 185:537 (1990); Kaufman *et al.*, EMBO J., 6:187 (1987). Accordingly, cells expressing high levels of the desired protein product may be obtained in a single step by culturing the initial transfectants in medium containing a selection agent appropriate for use with the particular selectable gene. However, the utility of these vectors is variable because of the unpredictable influence of the upstream product reading frame on selectable marker translation and because the upstream reading frame sometimes becomes deleted during methotrexate amplification (Kaufman *et al.*, J. Mol. Biol., 159:601-621 [1982]; Levinson, Methods in Enzymology, San Diego: Academic Press, Inc. [1990]). Later vectors incorporated an internal translation initiation site derived from members of the picornavirus family which is positioned between the product gene and the selectable gene (Pelletier *et al.*, Nature, 334:320 [1988]; Jang *et al.*, J. Virol., 63:1651 [1989]).

A third method for single-step selection involves use of a DNA construct with a selectable gene containing an intron within which is located a gene encoding the protein of interest. See U.S. Patent No. 5,043,270 and Abrams *et al.*, J. Biol. Chem., **264(24)**: 14016-14021 (1989). In yet another single-step selection method, host cells are co-transfected with an intron-modified selectable gene and a gene encoding the protein of interest. See WO 92/17566, published October 15, 1992. The intron-modified gene is prepared by inserting into the transcribed region of a selectable gene an intron of such length that the intron is correctly spliced from the corresponding mRNA precursor at low efficiency, so that the amount of selectable marker produced from the intron-modified selectable gene is substantially less than that produced from the starting selectable gene. These vectors help to insure the integrity of the integrated DNA construct, but transcriptional linkage is not achieved as selectable gene and the protein gene are driven by separate promoters.

Other mammalian expression vectors that have single transcription units have been described. Retroviral vectors have been constructed (Cepko *et al.*, Cell, 37:1053-1062 [1984]) in which a cDNA is inserted between the endogenous Moloney murine leukemia virus (M-MuLV) splice donor and splice acceptor sites which are followed by a neomycin resistance gene. This vector has been used to express a variety of gene products following retroviral infection of several cell types.

With the above drawbacks in mind, it is one object of the present invention to increase the level of homogeneity with regard to expression levels of stable clones transfected with a product gene of interest, by expressing a selectable marker (DHFR) and the protein of interest from a single promoter.

It is another object to provide a method for selecting stable, recombinant host cells that express high levels of a desired protein product, which method is rapid and convenient to perform, and reduces the numbers of transfected cells which need to be screened. Furthermore, it is

an object to allow high levels of single and two unit polypeptides to be rapidly generated from clones or pools of stable host cell transfectants.

It is an additional object to provide expression vectors which bias for active integration events (*i.e.* have an increased tendency to generate transformants wherein the DNA construct is inserted into a region of the genome of the host cell which results in high level expression of the product gene) and can accommodate a variety of product genes without the need for modification.

SUMMARY OF THE INVENTION

Accordingly, the present invention is directed to a DNA construct (DNA molecule) alternative terminology comprising a 5' transcriptional initiation site and a 3' transcriptional termination site, a selectable gene (preferably an amplifiable gene) and a product gene provided 3' to the selectable gene, a transcriptional regulatory region regulating transcription of both the selectable gene and the product gene, the selectable gene positioned within an intron defined by a splice donor site and a splice acceptor site. The splice donor site preferably comprises an effective splice donor sequence as herein defined and thereby regulates expression of the product gene using the transcriptional regulatory region.

In another embodiment, the invention provides a method for producing a product of interest comprising culturing a eukaryotic cell which has been transfected with the DNA construct described above, so as to express the product gene and recovering the product.

In a further embodiment, the invention provides a method for producing eukaryotic cells having multiple copies of the product gene comprising transfecting eukaryotic cells with the DNA construct described above (where the selectable gene is an amplifiable gene), growing the cells in a selective medium comprising an amplifying agent for a sufficient time for amplification to occur, and selecting cells having multiple copies of the product gene. Preferably transfection of the cells is achieved using electroporation.

After transfection of the host cells, most of the transfectants fail to exhibit the selectable phenotype characteristic of the protein encoded by the selectable gene, but surprisingly a small proportion of the transfectants do exhibit the selectable phenotype, and among those transfectants, the majority are found to express high levels of the desired product encoded by the product gene. Thus, the invention provides an improved method for the selection of recombinant host cells expressing high levels of a desired product, which method is useful with a wide variety of eukaryotic host cells and avoids the problems inherent in existing cell selection technology.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1D illustrate schematically various DNA constructs encompassed by the instant invention. The large arrows represent the selectable gene and the product gene, the V formed by the dashed lines shows the region of the precursor RNA internal to the 5' splice donor site (SD) and 3' splice acceptor site (SA) that is excised from vectors that contain a functional SD. The transcriptional regulatory region, selectable gene, product gene and transcriptional termination site are depicted in Figure 1A. Figure 1B depicts the DNA constructs of Example 1. The various splice donor sequences are depicted, *i.e.*, wild type ras splice donor sequence (WT ras), mutant ras splice donor sequence (MUTANT ras) and non-functional splice donor sequence (ΔGT). The probes used for Northern blot analysis in Example 1 are shown in Figure 1B. Figure 1C depicts the DNA constructs of Example 2 and Figure 1D depicts the DNA construct of Example 3 used for expression of anti-IgE V_H.

Figure 2 depicts schematically the control DNA construct used in Example 1.

Figures 3A-Q depict the nucleotide sequence (SEQ ID NO: 1) of the DHFR/intron-(WT ras SD)-tPA expression vector of Example 1.

Figure 4 is a bar graph which shows the number of colonies that form in selective medium after electroporation of linearized duplicate miniprep DNA's prepared in parallel from the three vectors shown in Figure 1B (*i.e.* with wild type ras splice donor sequence [WT ras], mutant ras splice donor sequence [MUTANT ras] and non-functional splice donor sequence [ΔGT]) and from the control vector that has DHFR under control of SV40 promoter and tPA under control of CMV promoter (see Figure 2). Cells were selected in nucleoside free medium and counted with an automated colony counter.

Figures 5A-C are bar graphs depicting expression of tPA from stable pools and clones generated from the vectors shown in Figure 1B. In Figure 5A greater than 100 clones from each vector transfection were mixed, plated in 24 well plates, and assayed by tPA ELISA at "saturation". In Figure 5B, twenty clones chosen at random derived from each of the vectors were assayed by tPA ELISA at "saturation". In Figure 5C, the pools mentioned in Figure 5A (except the ΔGT pool) were exposed to 200nM Mtx to select for DHFR amplification and then pooled and assayed for tPA expression.

Figures 6A-P depict the nucleotide sequence (SEQ ID NO: 2) of the DHFR/intron-(WT ras SD)-TNFr-IgG expression vector of Example 2.

Figures 7A-B are bar graphs depicting expression of TNFr-IgG using dicistronic or control vectors (see Example 2). Vectors containing TNFr-IgG (but otherwise identical to those described for tPA expression in Example 1) were constructed (see Figure 1C), introduced into dp12.CHO cells by electroporation, pooled, and assayed for product expression before (Figure 7A) and after (Figure 7B) being subjected to amplification in 200nM Mtx.

Figure 8 depicts schematically the DNA construct used for expression of the V_L of anti-IgE in Example 3.

Figures 9A-O depict the nucleotide sequence (SEQ ID NO: 3) of the anti-IgE V_H expression vector of Example 3.

Figures 10A-Q depict the nucleotide sequence (SEQ ID NO: 4) of the anti-IgE V_L expression vector of Example 3.

Figure 11 is a bar graph depicting anti-IgE expression in Example 3. Heavy (V_H) and light (V_L) chain expression vectors were constructed, co-electroporated into CHO cells, clones were selected and assayed for antibody expression. Additionally, pools were established and assessed with regard to expression before and after Mtx selection at 200nM and 1μM.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Definitions:

The "DNA construct" disclosed herein comprises a non-naturally occurring DNA molecule which can either be provided as an isolate or integrated in another DNA molecule *e.g.* in an expression vector or the chromosome of an eukaryotic host cell.

The term "selectable gene" as used herein refers to a DNA that encodes a selectable marker necessary for the growth or survival of a host cell under the particular cell culture conditions chosen. Accordingly, a host cell that is transformed with a selectable gene will be capable of growth or survival under certain cell culture conditions wherein a non-transfected host cell is not capable of growth or survival. Typically, a selectable gene will confer resistance to a drug or compensate for a metabolic or catabolic defect in the host cell. Examples of selectable genes are provided in the following table. See also Kaufman, Methods in Enzymology, 185: 537-566 (1990), for a review of these.

TABLE 1
Selectable Genes and their Selection Agents

Selection Agent	Selectable Gene
Methotrexate	Dihydrofolate reductase
Cadmium	Metallothionein
PALA	CAD
Xyl-A-or adenosine and 2'-deoxycoformycin	Adenosine deaminase
Adenine, azaserine, and coformycin	Adenylate deaminase
6-Azauridine, pyrazofuran	UMP Synthetase
Mycophenolic acid	IMP 5'-dehydrogenase

Mycophenolic acid with limiting xanthine	Xanthine-guanine phosphoribosyltransferase
Hypoxanthine, aminopterin, and thymidine (HAT)	Mutant HGPRTase or mutant thymidine kinase
5-Fluorodeoxyuridine	Thymidylate synthetase
Multiple drugs *e.g.* adriamycin, vincristine or colchicine	P-glycoprotein 170
Aphidicolin	Ribonucleotide reductase
Methionine sulfoximine	Glutamine synthetase
β-Aspartyl hydroxamate or Albizziin	Asparagine synthetase
Canavanine	Arginosuccinate synthetase
α-Difluoromethylornithine	Ornithine decarboxylase
Compactin	HMG-CoA reductase
Tunicamycin	*N*-Acetylglucosaminyl transferase
Borrelidin	Threonyl-tRNA synthetase
Ouabain	$Na^{+}K^{+}$-ATPase

The preferred selectable gene is an amplifiable gene. As used herein, the term "amplifiable gene" refers to a gene which is amplified (*i.e.* additional copies of the gene are generated which survive in intrachromosomal or extrachromosomal form) under certain conditions. The amplifiable gene usually encodes an enzyme (*i.e.* an amplifiable marker) which is required for growth of eukaryotic cells under those conditions. For example, the gene may encode DHFR which is amplified when a host cell transformed therewith is grown in Mtx. According to Kaufman, the selectable genes in Table 1 above can also be considered amplifiable genes. An example of a selectable gene which is generally not considered to be an amplifiable gene is the neomycin resistance gene (Cepko *et al.*, *supra*).

As used herein, "selective medium" refers to nutrient solution used for growing eukaryotic cells which have the selectable gene and therefore includes a "selection agent". Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ([MEM], Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ([DMEM], Sigma) are exemplary nutrient solutions. In addition, any of the media described in Ham and Wallace, Meth. Enz., 58:44 (1979), Barnes and Sato, Anal. Biochem., 102:255

(1980), U.S. Patent Nos. 4,767,704; 4,657,866; 4,927,762; or 4,560,655; WO 90/03430; WO 87/00195; U.S. Patent Re. 30,985; or U.S. Patent No. 5,122,469, the disclosures of all of which are incorporated herein by reference, may be used as culture media. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleosides (such as adenosine and thymidine), antibiotics (such as Gentamycin™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The preferred nutrient solution comprises fetal bovine serum.

The term "selection agent" refers to a substance that interferes with the growth or survival of a host cell that is deficient in a particular selectable gene. Examples of selection agents are presented in Table 1 above. The selection agent preferably comprises an "amplifying agent" which is defined for purposes herein as an agent for amplifying copies of the amplifiable gene, such as Mtx if the amplifiable gene is DHFR. See Table 1 for examples of amplifying agents.

As used herein, the term "transcriptional initiation site" refers to the nucleic acid in the DNA construct corresponding to the first nucleic acid incorporated into the primary transcript, *i.e.*, the mRNA precursor, which site is generally provided at, or adjacent to, the 5' end of the DNA construct.

The term "transcriptional termination site" refers to a sequence of DNA, normally represented at the 3' end of the DNA construct, that causes RNA polymerase to terminate transcription.

As used herein, "transcriptional regulatory region" refers to a region of the DNA construct that regulates transcription of the selectable gene and the product gene. The transcriptional regulatory region normally refers to a promoter sequence (*i.e.* a region of DNA involved in binding of RNA polymerase to initiate transcription) which can be constitutive or inducible and, optionally, an enhancer (*i.e.* a *cis*-acting DNA element, usually from about 10-300 bp, that acts on a promoter to increase its transcription).

As used herein, "product gene" refers to DNA that encodes a desired protein or polypeptide product. Any product gene that is capable of expression in a host cell may be used, although the methods of the invention are particularly suited for obtaining high-level expression of a product gene that is not also a selectable or amplifiable gene. Accordingly, the protein or polypeptide encoded by a product gene typically will be one that is not necessary for the growth or survival of a host cell under the particular cell culture conditions chosen. For example, product genes suitably encode a peptide, or may encode a polypeptide sequence of

amino acids for which the chain length is sufficient to produce higher levels of tertiary and/or quaternary structure.

Examples of bacterial polypeptides or proteins include, *e.g.*, alkaline phosphatase and β-lactamase. Examples of mammalian polypeptides or proteins include molecules such as renin; a growth hormone, including human growth hormone, and bovine growth hormone; growth hormone releasing factor; parathyroid hormone; thyroid stimulating hormone; lipoproteins; alpha-1-antitrypsin; insulin A-chain; insulin B-chain; proinsulin; follicle stimulating hormone; calcitonin; luteinizing hormone; glucagon; clotting factors such as factor VIIIC, factor IX, tissue factor, and von Willebrands factor; anti-clotting factors such as Protein C; atrial natriuretic factor; lung surfactant; a plasminogen activator, such as urokinase or human urine or tissue-type plasminogen activator (t-PA); bombesin; thrombin; hemopoietic growth factor; tumor necrosis factor-alpha and -beta; enkephalinase; RANTES (regulated on activation normally T-cell expressed and secreted); human macrophage inflammatory protein (MIP-1-alpha); a serum albumin such as human serum albumin; mullerian-inhibiting substance; relaxin A-chain; relaxin B-chain; prorelaxin; mouse gonadotropin-associated peptide; a microbial protein, such as beta-lactamase; DNase; inhibin; activin; vascular endothelial growth factor (VEGF); receptors for hormones or growth factors; integrin; protein A or D; rheumatoid factors; a neurotrophic factor such as bone-derived neurotrophic factor (BDNF), neurotrophin-3, -4, -5, or -6 (NT-3, NT-4, NT-5, or NT-6), or a nerve growth factor such as NGF-β; platelet-derived growth factor (PDGF); fibroblast growth factor such as aFGF and bFGF; epidermal growth factor (EGF); transforming growth factor (TGF) such as TGF-alpha and TGF-beta, including TGF-β1, TGF-β2, TGF-β3, TGF-β4, or TGF-β5; insulin-like growth factor-I and -II (IGF-I and IGF-II); des(1-3)-IGF-I (brain IGF-I), insulin-like growth factor binding proteins; CD proteins such as CD-3, CD-4, CD-8, and CD-19; erythropoietin; osteoinductive factors; immunotoxins; a bone morphogenetic protein (BMP); an interferon such as interferon-alpha, -beta, and -gamma; colony stimulating factors (CSFs), *e.g.*, M-CSF, GM-CSF, and G-CSF; interleukins (ILs), *e.g.*, IL-1 to IL-10; superoxide dismutase; T-cell receptors; surface membrane proteins; decay accelerating factor; viral antigen such as, for example, a portion of the AIDS envelope; transport proteins; homing receptors; addressins; regulatory proteins; antibodies; chimeric proteins such as immunoadhesins and fragments of any of the above-listed polypeptides.

The product gene preferably does not consist of an anti-sense sequence for inhibiting the expression of a gene present in the host. Preferred proteins herein are therapeutic proteins such as TGF-β, TGF-α, PDGF, EGF, FGF, IGF-I, DNase, plasminogen activators such as t-PA, clotting factors such as tissue factor and factor VIII, hormones such as relaxin and insulin, cytokines such as IFN-γ, chimeric proteins such as TNF receptor IgG immunoadhesin (TNFr-IgG) or antibodies such as anti-IgE.

The term "intron" as used herein refers to a nucleotide sequence present within the transcribed region of a gene or within a messenger RNA precursor, which nucleotide sequence is capable of being excised, or spliced, from the messenger RNA precursor by a host cell prior to translation. Introns suitable for use in the present invention are suitably prepared by any of several methods that are well known in the art, such as purification from a naturally occurring nucleic acid or *de novo* synthesis. The introns present in many naturally occurring eukaryotic genes have been identified and characterized. Mount, Nuc. Acids Res., **10**:459 (1982). Artificial introns comprising functional splice sites also have been described. Winey *et al.*, Mol. Cell Biol., **9**:329 (1989); Gatermann *et al.*, Mol. Cell Biol., **9**:1526 (1989). Introns may be obtained from naturally occurring nucleic acids, for example, by digestion of a naturally occurring nucleic acid with a suitable restriction endonuclease, or by PCR cloning using primers complementary to sequences at the 5' and 3' ends of the intron. Alternatively, introns of defined sequence and length may be prepared synthetically using various methods in organic chemistry. Narang *et al.*, Meth. Enzymol., **68**:90 (1979); Caruthers *et al.*, Meth. Enzymol., **154**:287 (1985); Froehler *et al.*, Nuc. Acids Res., **14**:5399 (1986).

As used herein "splice donor site" or "SD" refers to the DNA sequence immediately surrounding the exon-intron boundary at the 5' end of the intron, where the "exon" comprises the nucleic acid 5' to the intron. Many splice donor sites have been characterized and Ohshima *et al.*, J. Mol. Biol., **195**:247-259 (1987) provides a review of these. An "efficient splice donor sequence" refers to a nucleic acid sequence encoding a splice donor site wherein the efficiency of splicing of messenger RNA precursors having the splice donor sequence is between about 80 to 99% and preferably 90 to 95% as determined by quantitative PCR. Examples of efficient splice donor sequences include the wild type (WT) ras splice donor sequence and the GAC:GTAAGT sequence of Example 3. Other efficient splice donor sequences can be readily selected using the techniques for measuring the efficiency of splicing disclosed herein.

The terms "PCR" and "polymerase chain reaction" as used herein refer to the *in vitro* amplification method described in US Patent No. 4,683,195 (issued July 28, 1987). In general, the PCR method involves repeated cycles of primer extension synthesis, using two DNA primers capable of hybridizing preferentially to a template nucleic acid comprising the nucleotide sequence to be amplified. The PCR method can be used to clone specific DNA sequences from total genomic DNA, cDNA transcribed from cellular RNA, viral or plasmid DNAs. Wang & Mark, in PCR Protocols, pp.70-75 (Academic Press, 1990); Scharf, in PCR Protocols, pp. 84-98; Kawasaki & Wang, in PCR Technology, pp. 89-97 (Stockton Press, 1989). Reverse transcription-polymerase chain reaction (RT-PCR) can be used to analyze RNA samples containing mixtures of spliced and unspliced mRNA transcripts. Fluorescently tagged primers designed to span the intron are used to

amplify both spliced and unspliced targets. The resultant amplification products are then separated by gel electrophoresis and quantitated by measuring the fluorescent emission of the appropriate band(s). A comparison is made to determine the amount of spliced and unspliced transcripts present in the RNA sample.

One preferred splice donor sequence is a "consensus splice donor sequence". The nucleotide sequences surrounding intron splice sites, which sequences are evolutionarily highly conserved, are referred to as "consensus splice donor sequences". In the mRNAs of higher eukaryotes, the 5' splice site occurs within the consensus sequence AG:GUAAGU (wherein the colon denotes the site of cleavage and ligation). In the mRNAs of yeast, the 5' splice site is bounded by the consensus sequence :GUAUGU. Padgett, *et al.*, Ann. Rev. Biochem., 55:1119 (1986).

The expression "splice acceptor site" or "SA" refers to the sequence immediately surrounding the intron-exon boundary at the 3' end of the intron, where the "exon" comprises the nucleic acid 3' to the intron. Many splice acceptor sites have been characterized and Ohshima *et al.*, J. Mol. Biol., 195:247-259 (1987) provides a review of these. The preferred splice acceptor site is an efficient splice acceptor site which refers to a nucleic acid sequence encoding a splice acceptor site wherein the efficiency of splicing of messenger RNA precursors having the splice acceptor site is between about 80 to 99% and preferably 90 to 95% as determined by quantitative PCR. The splice acceptor site may comprise a consensus sequence. In the mRNAs of higher eukaryotes, the 3' splice acceptor site occurs within the consensus sequence $(U/C)_{11}$NCAG:G. In the mRNAs of yeast, the 3' acceptor splice site is bounded by the consensus sequence (C/U)AG:. Padgett, *et al.*, *supra*.

As used herein "culturing for sufficient time to allow amplification to occur" refers to the act of physically culturing the eukaryotic host cells which have been transformed with the DNA construct in cell culture media containing the amplifying agent, until the copy number of the amplifiable gene (and preferably also the copy number of the product gene) in the host cells has increased relative to the transformed cells prior to this culturing.

The term "expression" as used herein refers to transcription or translation occurring within a host cell. The level of expression of a product gene in a host cell may be determined on the basis of either the amount of corresponding mRNA that is present in the cell or the amount of the protein encoded by the product gene that is produced by the cell. For example, mRNA transcribed from a product gene is desirably quantitated by northern hybridization. Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, pp. 7.3-7.57 (Cold Spring Harbor Laboratory Press, 1989). Protein encoded by a product gene can be quantitated either by assaying for the biological activity of the protein or by employing assays that are independent of such activity, such as western blotting or radioimmunoassay using antibodies that are capable of reacting with the protein. Sambrook,

et al., Molecular Cloning: A Laboratory Manual, pp. 18.1-18.88 (Cold Spring Harbor Laboratory Press, 1989).

Modes for Carrying Out the Invention

Methods and compositions are provided for enhancing the stability and/or copy number of a transcribed sequence in order to allow for elevated levels of a RNA sequence of interest. In general, the methods of the present invention involve transfecting a eukaryotic host cell with an expression vector comprising both a product gene encoding a desired polypeptide and a selectable gene (preferably an amplifiable gene).

Selectable genes and product genes may be obtained from genomic DNA, cDNA transcribed from cellular RNA, or by *in vitro* synthesis. For example, libraries are screened with probes (such as antibodies or oligonucleotides of about 20-80 bases) designed to identify the selectable gene or the product gene (or the protein(s) encoded thereby). Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures as described in chapters 10-12 of Sambrook *et al.*, Molecular Cloning: A Laboratory Manual (New York: Cold Spring Harbor Laboratory Press, 1989). An alternative means to isolate the selectable gene or product gene is to use PCR methodology as described in section 14 of Sambrook *et al.*, *supra*.

A preferred method of practicing this invention is to use carefully selected oligonucleotide sequences to screen cDNA libraries from various tissues known to contain the selectable gene or product gene. The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized.

The oligonucleotide generally is labeled such that it can be detected upon hybridization to DNA in the library being screened. The preferred method of labeling is to use ^{32}P- labeled ATP with polynucleotide kinase, as is well known in the art, to radiolabel the oligonucleotide. However, other methods may be used to label the oligonucleotide, including, but not limited to, biotinylation or enzyme labeling.

Sometimes, the DNA encoding the selectable gene and product gene is preceded by DNA encoding a signal sequence having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the expression vector, or it may be a part of the selectable gene or product gene that is inserted into the expression vector. If a heterologous signal sequence is used, it preferably is one that is recognized and processed (*i.e.*, cleaved by a signal peptidase) by the host cell. For yeast secretion the native signal sequence may be substituted by, *e.g.*, the yeast invertase leader, alpha factor leader (including *Saccharomyces* and *Kluyveromyces* α-factor leaders, the latter described in U.S. Pat. No. 5,010,182 issued 23 April 1991), or acid phosphatase leader, the *C. albicans* glucoamylase leader (EP 362,179 published 4 April 1990), or the signal described in WO 90/13646 published 15 November 1990. In mammalian cell expression the native signal sequence

of the protein of interest is satisfactory, although other mammalian signal sequences may be suitable, such as signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders, for example, the herpes simplex gD signal. The DNA for such precursor region is ligated in reading frame to the selectable gene or product gene.

As shown in Figure 1A, the selectable gene is generally provided at the 5' end of the DNA construct and this selectable gene is followed by the product gene. Therefore, the full length (non-spiced) message will contain DHFR as the first open reading frame and will therefore generate DHFR protein to allow selection of stable transfectants. The full length message is not expected to generate appreciable amounts of the protein of interest as the second AUG in a dicistronic message is an inefficient initiator of translation in mammalian cells (Kozak, J. Cell Biol., 115: 887-903 [1991]).

The selectable gene is positioned within an intron. Introns are noncoding nucleotide sequences, normally present within many eukaryotic genes, which are removed from newly transcribed mRNA precursors in a multiple-step process collectively referred to as splicing.

A single mechanism is thought to be responsible for the splicing of mRNA precursors in mammalian, plant, and yeast cells. In general, the process of splicing requires that the 5' and 3' ends of the intron be correctly cleaved and the resulting ends of the mRNA be accurately joined, such that a mature mRNA having the proper reading frame for protein synthesis is produced. Analysis of a variety of naturally occurring and synthetically constructed mutant genes has shown that nucleotide changes at many of the positions within the consensus sequences at the 5' and 3' splice sites have the effect of reducing or abolishing the synthesis of mature mRNA. Sharp, Science, 235:766 (1987); Padgett, *et al.*, Ann. Rev. Biochem., 55:1119 (1986); Green, Ann. Rev. Genet., 20:671 (1986). Mutational studies also have shown that RNA secondary structures involving splicing sites can affect the efficiency of splicing. Solnick, Cell, 43:667 (1985); Konarska, *et al.*, Cell, 42:165 (1985).

The length of the intron may also affect the efficiency of splicing. By making deletion mutations of different sizes within the large intron of the rabbit beta-globin gene, Wieringa, *et al.* determined that the minimum intron length necessary for correct splicing is about 69 nucleotides. Cell, 37:915 (1984). Similar studies of the intron of the adenovirus E1A region have shown that an intron length of about 78 nucleotides allows correct splicing to occur, but at reduced efficiency. Increasing the length of the intron to 91 nucleotides restores normal splicing efficiency, whereas truncating the intron to 63 nucleotides abolishes correct splicing. Ulfendahl, *et al.*, Nuc. Acids Res., 13:6299 (1985).

To be useful in the invention, the intron must have a length such that splicing of the intron from the mRNA is efficient. The preparation of introns of differing lengths is a routine matter, involving methods well known in the art, such as *de novo* synthesis or *in vitro* deletion

mutagenesis of an existing intron. Typically, the intron will have a length of at least about 150 nucleotides, since introns which are shorter than this tend to be spliced less efficiently. The upper limit for the length of the intron can be up to 30 kB or more. However, as a general proposition, the intron is generally less than about 10 kB in length.

The intron is modified to contain the selectable gene not normally present within the intron using any of the various known methods for modifying a nucleic acid *in vitro*. Typically, a selectable gene will be introduced into an intron by first cleaving the intron with a restriction endonuclease, and then covalently joining the resulting restriction fragments to the selectable gene in the correct orientation for host cell expression, for example by ligation with a DNA ligase enzyme.

The DNA construct is dicistronic, *i.e.* the selectable gene and product gene are both under the transcriptional control of a single transcriptional regulatory region. As mentioned above, the transcriptional regulatory region comprises a promoter. Suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase (Hitzeman *et al.*, J. Biol. Chem., 255:2073 [1980]) or other glycolytic enzymes (Hess *et al.*, J. Adv. Enzyme Reg., 7:149 [1968]; and Holland, Biochemistry, 17:4900 [1978]), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in Hitzeman *et al.*, EP 73,657A. Yeast enhancers also are advantageously used with yeast promoters.

Expression control sequences are known for eukaryotes. Virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CXCAAT region where X may be any nucleotide.

Product gene transcription from vectors in mammalian host cells is controlled by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g. the actin promoter or an immunoglobulin promoter, from heat-shock promoters, and from the promoter normally associated with the product gene, provided such promoters are compatible with the host cell systems.

The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. Fiers *et al.*, Nature, 273:113 (1978); Mulligan and Berg, Science, 209:1422-1427 (1980); Pavlakis *et al.*, Proc. Natl. Acad. Sci. USA, 78:7398-7402 (1981). The immediate early promoter of the human cytomegalovirus (CMV) is conveniently obtained as a *Hin*dIII E restriction fragment. Greenaway *et al.*, Gene, 18:355-360 (1982). A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. 4,419,446. A modification of this system is described in U.S. 4,601,978. See also Gray *et al.*, Nature, 295:503-508 (1982) on expressing cDNA encoding immune interferon in monkey cells; , Reyes *et al.*, Nature, 297:598-601 (1982) on expression of human β-interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus, Canaani and Berg, Proc. Natl. Acad. Sci. USA, 79:5166-5170 (1982) on expression of the human interferon β1 gene in cultured mouse and rabbit cells, and Gorman *et al.*, Proc. Natl. Acad. Sci. USA, 79:6777-6781 (1982) on expression of bacterial CAT sequences in CV-1 monkey kidney cells, chicken embryo fibroblasts, Chinese hamster ovary cells, HeLa cells, and mouse NIH-3T3 cells using the Rous sarcoma virus long terminal repeat as a promoter.

Preferably the transcriptional regulatory region in higher eukaryotes comprises an enhancer sequence. Enhancers are relatively orientation and position independent having been found 5' (Lainins *et al.*, Proc. Natl. Acad. Sci. USA, 78:993 [1981]) and 3' (Lusky *et al.*, Mol. Cell Bio., 3:1108 [1983]) to the transcription unit, within an intron (Banerji *et al.*, Cell, 33:729 [1983]) as well as within the coding sequence itself (Osborne *et al.*, Mol. Cell Bio., 4:1293 [1984]). Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer (CMV), the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, Nature, 297:17-18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the product gene, but is preferably located at a site 5' from the promoter.

The DNA construct has a transcriptional initiation site following the transcriptional regulatory region and a transcriptional termination region following the product gene (see Figure 1A). These sequences are provided in the DNA construct using techniques which are well known in the art.

The DNA construct normally forms part of an expression vector which may have other components such as an origin of replication (*i.e.*, a nucleic acid sequence that enables the vector to replicate in one or more selected host cells) and, if desired, one or more additional selectable gene(s). Construction of suitable vectors containing the desired coding and control sequences employs standard ligation techniques. Isolated plasmids or DNA

fragments are cleaved, tailored, and religated in the form desired to generate the plasmids required.

Generally, in cloning vectors the origin of replication is one that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known. The 2μ plasmid origin of replication is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter).

Most expression vectors are "shuttle" vectors, *i.e.*, they are capable of replication in at least one class of organisms but can be transfected into another organism for expression. For example, a vector is cloned in *E. coli* and then the same vector is transfected into yeast or mammalian cells for expression even though it is not capable of replicating independently of the host cell chromosome.

For analysis to confirm correct sequences in plasmids constructed, plasmids from the transformants are prepared, analyzed by restriction, and/or sequenced by the method of Messing *et al.*, Nucleic Acids Res., 9:309 (1981) or by the method of Maxam *et al.*, Methods in Enzymology, 65:499 (1980).

The expression vector having the DNA construct prepared as discussed above is transformed into a eukaryotic host cell. Suitable host cells for cloning or expressing the vectors herein are yeast or higher eukaryote cells.

Eukaryotic microbes such as filamentous fungi or yeast are suitable hosts for vectors containing the product gene. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *S. pombe* [Beach and Nurse, Nature, 290:140 (1981)], *Kluyveromyces lactis* [Louvencourt *et al.*, J. Bacteriol., 737 (1983)], *yarrowia* [EP 402,226], *Pichia pastoris* [EP 183,070], *Trichoderma reesia* [EP 244,234], *Neurospora crassa* [Case *et al.*, Proc. Natl. Acad. Sci. USA, 76:5259-5263 (1979)], and *Aspergillus* hosts such as *A. nidulans* [Ballance *et al.*, Biochem. Biophys. Res. Commun., 112:284-289 (1983); Tilburn *et al.*, Gene, 26:205-221 (1983); Yelton *et al.*, Proc. Natl. Acad. Sci. USA, 81:1470-1474 (1984)] and *A. niger* [Kelly and Hynes, EMBO J., 4:475-479 (1985)].

Suitable host cells for the expression of the product gene are derived from multicellular organisms. Such host cells are capable of complex processing and glycosylation activities. In principle, any higher eukaryotic cell culture is workable, whether from vertebrate or invertebrate culture. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda*

(caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosphila melanogaster* (fruitfly), and *Bombyx mori* host cells have been identified. See, e.g., Luckow *et al.*, Bio/Technology, 6:47-55 (1988); Miller *et al.*, in Genetic Engineering, Setlow, J.K. *et al.*, eds., Vol. 8 (Plenum Publishing, 1986), pp. 277-279; and Maeda *et al.*, Nature, 315:592-594 (1985). A variety of such viral strains are publicly available, e.g., the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells.

Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can be utilized as hosts. Typically, plant cells are transfected by incubation with certain strains of the bacterium *Agrobacterium tumefaciens*, which has been previously manipulated to contain the product gene. During incubation of the plant cell culture with *A. tumefaciens*, the product gene is transferred to the plant cell host such that it is transfected, and will, under appropriate conditions, express the product gene. In addition, regulatory and signal sequences compatible with plant cells are available, such as the nopaline synthase promoter and polyadenylation signal sequences. Depicker *et al.*, J. Mol. Appl. Gen., 1:561 (1982). In addition, DNA segments isolated from the upstream region of the T-DNA *780* gene are capable of activating or increasing transcription levels of plant-expressible genes in recombinant DNA-containing plant tissue. EP 321,196 published 21 June 1989.

However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure in recent years [Tissue Culture, Academic Press, Kruse and Patterson, editors (1973)]. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, J. Gen Virol., 36:59 [1977]); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub and Chasin, Proc. Natl. Acad. Sci. USA, 77:4216 [1980]); dp12.CHO cells (EP 307,247 published 15 March 1989); mouse sertoli cells (TM4, Mather, Biol. Reprod., 23:243-251 [1980]); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather *et al.*, Annals N.Y. Acad. Sci., 383:44-68 [1982]); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

Host cells are transformed with the above-described expression or cloning vectors of this invention and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw *et al.*, Gene, 23:315 (1983) and WO 89/05859 published 29 June 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb, Virology, 52:456-457 (1978) may be used. General aspects of mammalian cell host system transformations have been described by Axel in U.S. 4,399,216 issued 16 August 1983. Transformations into yeast are typically carried out according to the method of Van Solingen *et al.*, J. Bact., 130:946 (1977) and Hsiao *et al.*, Proc. Natl. Acad. Sci. (USA), 76:3829 (1979). However, other methods for introducing DNA into cells such as by nuclear injection or by protoplast fusion may also be used.

In the preferred embodiment the DNA is introduced into the host cells using electroporation. See Andreason, J. Tiss. Cult. Meth., 15:56-62 (1993), for a review of electroporation techniques useful for practicing the instantly claimed invention. It was discovered that electroporation techniques for introducing the DNA construct into the host cells were preferable over calcium phosphate precipitation techniques insofar as the latter could cause the DNA to break up and forming concantemers.

The mammalian host cells used to express the product gene herein may be cultured in a variety of media as discussed in the definitions section above. The media contains the selection agent used for selecting transformed host cells which have taken up the DNA construct (either as an intra- or extra-chromosomal element). To achieve selection of the transformed eukaryotic cells, the host cells may be grown in cell culture plates and individual colonies expressing the selectable gene (and thus the product gene) can be isolated and grown in growth medium until the nutrients are depleted. The host cells are then analyzed for transcription and/or transformation as discussed below. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA (Thomas, Proc. Natl. Acad. Sci. USA, 77:5201-5205 [1980]), dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Various labels may be employed, most commonly radioisotopes, particularly ^{32}P. However, other techniques may also be employed, such as using biotin-modified nucleotides for introduction into a polynucleotide. The biotin then serves as the site for binding to avidin or antibodies, which may be labeled with a wide variety of labels, such as radionuclides, fluorescens, enzymes, or the like. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the

formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. With immunohistochemical staining techniques, a cell sample is prepared, typically by dehydration and fixation, followed by reaction with labeled antibodies specific for the gene product coupled, where the labels are usually visually detectable, such as enzymatic labels, fluorescent labels, luminescent labels, and the like. A particularly sensitive staining technique suitable for use in the present invention is described by Hsu *et al.*, Am. J. Clin. Path., 75:734-738 (1980).

In the preferred embodiment, the mRNA is analyzed by quantitative PCR (to determine the efficiency of splicing) and protein expression is measured using ELISA as described in Example 1 herein.

The product of interest preferably is recovered from the culture medium as a secreted polypeptide, although it also may be recovered from host cell lysates when directly expressed without a secretory signal. When the product gene is expressed in a recombinant cell other than one of human origin, the product of interest is completely free of proteins or polypeptides of human origin. However, it is necessary to purify the product of interest from recombinant cell proteins or polypeptides to obtain preparations that are substantially homogeneous as to the product of interest. As a first step, the culture medium or lysate is centrifuged to remove particulate cell debris. The product of interest thereafter is purified from contaminant soluble proteins and polypeptides, for example, by fractionation on immunoaffinity or ion-exchange columns; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel electrophoresis using, for example, Sephadex G-75; chromatography on plasminogen columns to bind the product of interest and protein A Sepharose columns to remove contaminants such as IgG.

The following examples are offered by way of illustration only and are not intended to limit the invention in any manner. All patent and literature references cited herein are expressly incorporated by reference.

EXAMPLE 1

tPA production using the dicistronic expression vectors

It was sought to increase the level of homogeneity with regard to expression levels of stable clones by expressing a selectable marker (such as DHFR) and the protein of interest from a single promoter. These vectors divert most of the transcript to product expression while linking it at a fixed ratio to DHFR expression via differential splicing.

Vectors were constructed which were derived from the vector pRK (Suva *et al.*, Science, 237:893-896 [1987]) which contains an intron between the cytomegalovirus immediate early promoter (CMV) and the cDNA that encodes

the polypeptide of interest. The intron of pRK is 139 nucleotides in length, has a splice donor site derived from cytomegalovirus immediate early gene (CMVIE), and a splice acceptor site from an IgG heavy chain variable region (V_H) gene (Eaton et al., Biochem., 25:8343 [1986]).

DHFR/intron vectors were constructed by inserting an EcoRV linker into the BSTX1 site present in the intron of pRK7. An 830 base-pair fragment containing a mouse DHFR coding fragment was inserted to obtain DHFR intron expression vectors which differ only in the sequence that comprises the splice donor site. Those sequences were altered by overlapping PCR mutagenesis to obtain sequences that match splice donor sites found between exons 3 and 4 of normal and mutant Ras genes. PCR was also used to destroy the splice donor site.

A mouse DHFR cDNA fragment (Simonsen et al., Proc. Natl. Acad. Sci. USA, 80:2495-2499 [1983]) was inserted into the intron of this vector 59 nucleotides downstream of the splice donor site. The splice donor site of this vector was altered by mutagenesis to change the ratio of spliced to non-spliced message in transfected cells. It has previously been shown that a single nucleotide change (G to A) converted a relatively efficient splice donor site found in the normal ras gene into an inefficient splice site (Cohen *et al.*, Nature, 334:119-124 [1988]). This effect has been demonstrated in the context of the ras gene and confirmed when these sequences were transferred to human growth hormone constructs (Cohen *et al.*, Cell, 58:461-472 [1989]). Additionally, a non functional 5' splice site (GT to CA) was constructed as a control (ΔGT). A polylinker was inserted 35 nucleotides downstream of the 3' splice site to accept the cDNA of interest. A vector containing tPA (Pennica et al., Nature, 301:214-221 [1983]) was linearized downstream of the polyadenylation site before it was introduced into CHO cells (Potter *et al.*, Proc. Natl. Acad. Sci. USA, 81:7161 [1984]).

Plasmid DNA's that contained DHFR/intron, tPA and (a) wild type ras (WT ras), *i.e.* Figure 3 (SEQ ID NO: 1), (b) mutant ras, or (c) non-functional splice donor site (ΔGT) were introduced into CHO DHFR minus cells by electroporation. The intron vectors were each linearized downstream of the polyadenylation site by restriction endonuclease treatment. The control vector was linearized downstream of the second polyadenylation site. The DNA's were ethanol precipitated after phenol/chloroform extraction and were resuspended in 20μl 1/10 Tris EDTA. Then, 10μg of DNA was incubated with 10^7 CHO.dp12 cells (EP 307,247 published 15 March 1989) in 1 ml of PBS on ice for 10 min. before electroporation at 400 volts and 330μf using a BRL Cell Porator.

Cells were returned to ice for 10 min. before being plated into non-selective medium. After 24 hours cells were fed nucleoside-free medium to select for stable DHFR+ clones which were pooled. The pooled DHFR+ clones were lysed and mRNA's were prepared.

To prepare the mRNA, RNA was extracted from 5 x 10^7 cells which were grown from pools of more than 200 clones derived from the stable

transfection of the three vectors, the essential construction of which is shown in Figure 1B and from non-transfected CHO cells. RNA was purified over oligo-DT cellulase (Collaborative Biomedical Products). 10μg of mRNA was then subjected to Northern blotting which involved running the mRNA on a 1.2% agarose, 6.6% formaldehyde gel, and transferring it to a nylon filter (Stratagene Duralon-UV membrane), prehybridized, probed and washed according to the manufacturer's instructions.

The filter was probed sequentially using probes (shown in Figure 1B) that would detect (a) the full length message, (b) both full length and spliced message, or (c) beta actin. Probing with the long probe showed that the vector that contains the efficient splice donor site (*i.e.* WT ras) generates predominately a mRNA of the size predicted for the spliced product while the other two vectors gave rise primarily to a mRNA that corresponds in size to non-spliced message. The DHFR probe detected only full length message and demonstrated that the WT ras splice donor derived vector generates very little full length message with which to confer a DHFR positive phenotype.

Figure 4 shows the number of DHFR positive colonies obtained after duplicate electroporations with the three intron vectors described above and from a conventional vector that has a CMV promoter driving tPA and a SV40 promoter driving DHFR (see Figure 2). The increase in colony number parallels the increase in full length message that accumulates with the modification of the splice donor sites. The conventional vector efficiently generates colonies and does not vary significantly from the ΔGT construct.

The level of tPA expression was determined by seeding cells in 1 ml of F12:DMEM (50:50, with 5% FBS) in 24 well dishes to near confluency. Growth of the cells continued until the media was exhausted. Media was then assayed by ELISA for tPA production. Briefly, anti-tPA antibody was coated onto the wells of an ELISA microtiter plate, media samples were added to the wells followed by washing. Binding of the antigen (tPA) was then quantified using horse radish peroxidase (HRPO) labelled anti-tPA antibody.

Figure 5A depicts the titers of secreted tPA protein after pooling the clones of each group shown in Figure 4. While the number of colonies increased with a weakening of splice donor function, the inverse was seen with respect to tPA expression. The expression levels are consistent with the RNA products that are observed; as more of the dicistronic message is spliced an increased amount of message will contain tPA as the first open reading frame resulting in increased tPA expression. A mutation of GT to CA in the splice donor site results in an abundance of DHFR positive colonies which express undetectable levels of tPA, possibly resulting from inefficient utilization of the second AUG. Importantly, Figure 5A also shows that expression levels obtained from one of the dicistronic vectors (with WT ras SD) was about threefold higher than that obtained with the control vector containing a CMV promoter/enhancer driving tPA, SV40

promoter/enhancer controlling DHFR and SV40 polyadenylation signals controlling the expression of tPA and DHFR.

Additionally, the homogeneity of expression in the pools was investigated. Figure 5B shows that all 20 clones generated by the WT ras splice donor site derived dicistronic vectors express detectable levels of tPA while only 4 of 20 clones generated by the control vector express tPA. None of the clones transfected with the non-splicing (ΔGT) vector expressed tPA levels detectable by ELISA. This finding is consistent with previous observations that relatively few clones generated by conventional vectors make useful levels of protein.

Expression of tPA was increased following methotrexate amplification of pools. Figure 5C shows that 2 of the dicistronic vector derived pools (*i.e.* with WT ras and MUTANT ras SD sites) increased in expression markedly (8.4 and 7.7 fold), while the pool generated by the conventional vector increased only slightly (2.8 fold) when each was subjected to 200 nM Mtx. An overall increase of 9 fold was obtained using the best dicistronic (WT ras SD) versus the conventional vector following amplification. Growth of the highest expressing amplified pool in nutrient rich production medium yielded titers of 4.2 μg/ml tPA.

It was shown that manipulation of the splice donor sequence alters the ratio of spliced to full length message and the number of colonies that form in selective medium. It was also shown that dicistronic expression vectors generate clones that express high levels of recombinant proteins. Surprisingly, it was possible to isolate high expressors which had the efficient WT ras splice donor site by selection for DHFR$^+$ cells despite the efficiency with which the DHFR gene was spliced from the RNA precursors formed in these cells.

EXAMPLE 2

TNFr-IgG production using the dicistronic expression vectors

To prove the general applicability of this approach, a second product was evaluated in the dicistronic vector system containing, as the DNA of interest, an immunoadhesin (TNFr-IgG) capable of binding tumor necrosis factor (TNF) (Ashkenazi *et al.*, Proc. Natl. Acad. Sci. USA, 88:10535-10539 [1991]). The experiments described in Example 1 above were essentially repeated except that the product gene encoded the immunoadhesin TNFr-IgG. Plasmid DNA's that contained a TNFr-IgG cDNA and (a) WT ras, *i.e.* Figure 6 (SEQ ID NO: 2), (b) mutant ras or (c) nonfunctional splice donor site (ΔGT) were introduced into the dp12.CHO cells as discussed for Example 1. See Figure 1C for an illustration of the DNA constructs.

It was discovered that the number of DHFR positive colonies generated by three of these vectors was similar to that seen with the tPA constructs. Expression of TNFr-IgG also paralleled that seen with the tPA constructs (Figure 7A). Amplification of pools from two of the constructs showed a marked increase in expression of immunoadhesin (9.6 and 6.8 fold) (Figure

7B). The best of these amplified pools expressed 9.5 μg/ml when grown in nutrient rich production medium.

Thus, it was again shown that dicistronic expression vectors generate clones that express high levels of recombinant proteins. Furthermore, contrary to expectations, it was discovered that isolation of high product expressing host DHFR⁺ cells was possible using an efficient splice donor site (*i.e.* the WT ras splice donor site).

EXAMPLE 3

Antibody production using a dicistronic expression vector

The usefulness of this system for antibody expression was evaluated by testing production of an antibody directed against IgE (Presta *et al.*, Journal of Immunology, 151:2623-2632 [1993]). Further, the flexibility of the system with regard to transcription initiation was tested by replacing the CMV promoter/enhancer present in the previous vectors with the promoter/ enhancer derived from the early region of SV40 virus (Griffin, B., Structure and Genomic Organization of SV40 and Polyoma Virus, In J. Tooze [Ed] DNA Tumor Viruses, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). The heavy chain of the antibody was inserted downstream of DHFR as described in the earlier tPA and TNFr-IgG constructs. Additionally, a new splice donor site sequence (GAC:GTAAGT) was engineered into the vector which matches the consensus splice donor site more closely than did the splice donor sites present in the vectors tested in Examples 1 and 2. The resultant expression vector is shown in Figures 1D and 9.

It was discovered that this vector produced fewer colonies than the vectors previously tested, and produced predominantly a spliced RNA product. A second vector was constructed to have the light chain of the antibody under control of the SV40 promoter/enhancer and poly-A and the hygromycin B resistance gene under control of the CMV promoter/enhancer and SV40 poly-A. These vectors were linearized at unique HpaI sites downstream of the poly-A signal, mixed at a ratio of light chain vector to heavy chain vector of 10:3 and electroporated into CHO cells using an optimized protocol (as discussed in Examples 1 and 2).

Figure 11 shows the levels of antibody expressed by clones and pools after selection in hygromycin B followed by selection for DHFR expression. All 20 of the clones analyzed expressed high levels of antibody when grown in rich medium and varied from one another by only a factor of four. A pool of antibody producing clones was generated and assayed shortly after it was established. That pool was grown continuously for 6 weeks without a significant decrease in productivity demonstrating that its stability was sufficient to generate gram quantities of protein from its large scale culture.

The pool was subjected to methotrexate amplification at 200nM and 1μM and achieved a greater than 2 fold increase in antibody titer. The 1μM Mtx resistant pool achieved a titer of 41 mg/L when grown under optimal conditions in suspension culture.

The structure of the expressed antibody was examined. Proteins expressed by the 200nM methotrexate resistant pool and by a well characterized expression clone generated by conventional vectors (Presta *et al*. [1993], *supra*) were metabolically labeled with S^{35} cysteine and methionine. In particular, confluent 35mm plates of cells were metabolically labeled with 50μCi each S-35 methionine and S-35 cysteine (Amersham) in serum free cysteine and methionine free F12:DMEM. After one hour, nutrient rich production media was added and labeled proteins were allowed to "chase" into the medium for six more hours. Proteins were run on a 12% SDS/PAGE gel (NOVEX) non-reduced or following reduction with B-mercaptoethanol. Dried gels were exposed to film for 16 hours. CHO control cells were also labeled.

The majority of the antibody protein is secreted with a molecular weight of about 155 kilodaltons, consistent with a properly disulfide-linked antibody molecule with 2 light and 2 heavy chains. Upon reduction the molecular weight shifts to 2 approximately equally abundant proteins of 22.5 and 55 kilodaltons. The protein generated from the pool is indistinguishable from the antibody produced by the well characterized expression clone, with no apparent increase of free heavy or light chain expressed by the pool.

CONCLUSION

The efficient expression system described herein utilizes vectors consisting of promoter/enhancer elements followed by an intron containing the selectable marker coding sequence, followed by the cDNA of interest and a polyadenylation signal.

Several splice donor site sequences were tested for their effect on colony number and expression of the cDNA of interest. A non-functional splice donor site, splice donor sites found in an intron between exons 3 and 4 of mutant (mutant ras) and normal (WT ras) forms of the Harvey Ras gene and another efficient SD site (see Example 3) were used. The vectors were designed to direct expression of dicistronic primary transcripts. Within a transfected cell some of the transcripts remain full length while the remainder are spliced to excise the DHFR coding sequence. When the splice donor site is weakened or destroyed an increase in colony number is observed.

Expression levels show the inverse pattern, with the most efficient splice donor sites generating the highest levels of tPA, TNFr immunoadhesin or anti-IgE V_H.

The homogeneity of expression of clones generated by the ras splice donor site intron DHFR vectors was compared to clones generated from a conventional vector with a separate promoter/enhancer and polyadenylation signal for each DHFR and tPA. The DHFR intron vector gives rise to colonies that are much more homogeneous with regard to expression than those generated by the conventional vector. Non-expressing clones derived from the conventional vector may be the result of breaks in the tPA or

TNFr-IgG domain of the plasmid during integration into the genome or the result of methylation of promoter elements (Busslinger *et al.*, Cell, 34:197-206 [1983]; Watt *et al.*, Genes and Development, 2:1136-1143 [1988]) driving tPA or TNFr-IgG expression. Promoter silencing by methylation or breaks in the DHFR-intron vectors would very likely render them incapable of conferring a DHFR positive phenotype.

It was found that pools generated by the DHFR-intron vectors could be amplified in methotrexate and would increase in expression by a factor of 8.4 (tPA), or 9.8 (TNFr-IgG). Pools from conventional vectors increased by only 2.8 and 3.0 fold for tPA and TNFr-IgG when amplified similarly. Amplified pools resulted in 9 fold higher tPA levels and 15 fold higher TNFr-IgG levels when compared to the conventional vector amplified pools.

Without being limited to any theory, the increase in expression of methotrexate resistant pools derived from the dicistronic vectors is likely due to the transcriptional linkage of DHFR and the product; when cells are selected for increased DHFR expression they consistently over-express product. Conventional approaches lack selectable marker and cDNA expression linkage and therefore methotrexate amplification often generates DHFR overexpression without the concomitant increase in product expression.

A further increase of 4 and 6.3 fold in expression were obtained when amplified tPA and TNFr-IgG pools were transferred from the media used for the selections and amplifications to a nutrient rich production medium.

In Example 3, the expression vector had a splice donor site that more closely matches the consensus splice donor sequence and had the heavy chain of a humanized anti-IgE antibody inserted downstream. This vector was linearized and co-electroporated with a second linearized vector that expresses the hygromycin resistance gene and the light chain of the antibody each under the control of its own promoter/enhancer and poly-A signals. An excess of light chain expression vector over the heavy chain dicistronic expression vector was used to bias in favor of light chain expression. Clones and a pool were generated after hygromycin B and DHFR selections. The clones were found to express relatively consistent, high levels of antibody, as did the pool. The 1μM pool achieved a titer of 41mg/L when grown under optimal conditions in suspension culture.

The anti-IgE antibody was assessed by metabolic labeling followed by SDS/PAGE under reducing and non reducing conditions and found to be indistinguishable from the protein expressed by a highly characterized clonal cell line. Of particular importance is the finding that no free light chain is observed in the pool relative to the clone.

A stable expression system for CHO cells has been developed that produces high levels of recombinant proteins rapidly and with less effort than that required by other expression systems. The vector system generates stable clones that express consistently high levels thereby reducing the number of clones that must be screened to obtain a highly productive clonal line. Alternatively, pools have been used to conveniently generate moderate to high levels of protein. This approach

may be particularly useful when a number of related proteins are to be expressed and compared.

Without being limited to this theory, it is possible the vectors that have very efficient splice donor sites generate very productive clones because so little transcript remains non spliced that only integration events that lead to the generation of high levels of RNA produce enough DHFR protein to give rise to colonies in selective medium. The high level of spliced message from such clones is then translated into abundant amounts of the protein of interest. Pools of clones made concurrently by introducing conventional vectors expressed lower levels of protein, and were unstable with regard to long term expression, and expression could not be appreciably increased when the cells were subjected to methotrexate amplification.

The system developed herein is versatile in that it allows high levels of single and multiple subunit polypeptides to be rapidly generated from clones or pools of stable transfectants. This expression system combines the advantages of transient expression systems (rapid and labor non intensive generation of research amounts of protein) with the concurrent development of highly productive stable production cell lines.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: GENENTECH, INC.

(ii) TITLE OF INVENTION: METHOD FOR SELECTING HIGH-EXPRESSING HOST CELLS

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
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(C) CITY: South San Francisco
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 94080

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 5.25 inch, 360 Kb floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: patin (Genentech)

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/286740
(B) FILING DATE: 05-AUG-1994

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7360 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT 50

TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC 100

TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG 150

ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA 200

TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC 250

ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT 300

AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC 350

TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC 400

GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA 450

TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA 500

AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC 550

AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT 600

TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT 650

CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA 700

TTGGAACGCG GATTCCCCGT GCCAAGAGTG CTGTAAGTAC CGCCTATAGA 750

GCGATAAGAG GATTTTATCC CCGCTGCCAT CATGGTTCGA CCATTGAACT 800

GCATCGTCGC CGTGTCCCAA AATATGGGGA TTGGCAAGAA CGGAGACCTA 850

CCCTGCCCTC CGCTCAGGAA CGCGTTCAAG TACTTCCAAA GAATGACCAC 900

AACCTCTTCA GTGGAAGGTA AACAGAATCT GGTGATTATG GGTAGGAAAA 950

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AGATGCTTTC AAGTTCTCTG CTCCCCTCCT AAAGCTATGC ATTTTTATAA 1400

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GGCTACAATT AATACATAAC CTTATGTATC ATACACATAG ATTTAGGTGA 1500

CACTATAGAA TAACATCCAC TTTGCCTTTC TCTCCACAGG TGTCACTCCA 1550

GGTCAACTGC ACCTCGGTTC TAAGCTTGGG CTGCAGGTCG CCGTGAATTT 1600

AAGGGACGCT GTGAAGCAAT CATGGATGCA ATGAAGAGAG GGCTCTGCTG 1650

TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT TTCGCCCAGC CAGGAAATCC 1700

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GCTCAGAAGC AACCGGGTGG AATATTGCTG GTGCAACAGT GGCAGGGCAC 1850

AGTGCCACTC AGTGCCTGTC AAAAGTTGCA GCGAGCCAAG GTGTTTCAAC 1900

GGGGGCACCT GCCAGCAGGC CCTGTACTTC TCAGATTTCG TGTGCCAGTG 1950

CCCCGAAGGA TTTGCTGGGA AGTGCTGTGA AATAGATACC AGGGCCACGT 2000

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CCATCTTTGC CAAGCACAGG AGGTCGCCCG GAGAGCGGTT CCTGTGCGGG 2650

GGCATACTCA TCAGCTCCTG CTGGATTCTC TCTGCCGCCC ACTGCTTCCA 2700

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GGGTGGTCCC TGGCGAGGAG GAGCAGAAAT TTGAAGTCGA AAAATACATT 2800

GTCCATAAGG AATTCGATGA TGACACTTAC GACAATGACA TTGCGCTGCT 2850

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TCTCCAGACC CACCACACCG CAGAAGCGGG ACGAGACCCT ACAGGAGAGG 3450

GAAGAGTGCA TTTTCCCAGA TACTTCCCAT TTTGGAAGTT TTCAGGACTT 3500

GGTCTGATTT CAGGATACTC TGTCAGATGG GAAGACATGA ATGCACACTA 3550

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GATGGCCGCC ATGGCCCAAC TTGTTTATTG CAGCTTATAA TGGTTACAAA 3650

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TTCTAGTTGT GGTTTGTCCA AACTCATCAA TGTATCTTAT CATGTCTGGA 3750

TCGATCGGGA ATTAATTCGG CGCAGCACCA TGGCCTGAAA TAACCTCTGA 3800

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TTACATCGAA CTGGATCTCA ACAGCGGTAA GATCCTTGAG AGTTTTCGCC 5500

CCGAAGAACG TTTTCCAATG ATGAGCACTT TTAAAGTTCT GCTATGTGGC 5550

GCGGTATTAT CCCGTGATGA CGCCGGGCAA GAGCAACTCG GTCGCCGCAT 5600

ACACTATTCT CAGAATGACT TGGTTGAGTA CTCACCAGTC ACAGAAAAGC 5650

ATCTTACGGA TGGCATGACA GTAAGAGAAT TATGCAGTGC TGCCATAACC 5700

ATGAGTGATA ACACTGCGGC CAACTTACTT CTGACAACGA TCGGAGGACC 5750

GAAGGAGCTA ACCGCTTTTT TGCACAACAT GGGGGATCAT GTAACTCGCC 5800

TTGATCGTTG GGAACCGGAG CTGAATGAAG CCATACCAAA CGACGAGCGT 5850

GACACCACGA TGCCAGCAGC AATGGCAACA ACGTTGCGCA AACTATTAAC 5900

TGGCGAACTA CTTACTCTAG CTTCCCGGCA ACAATTAATA GACTGGATGG 5950

AGGCGGATAA AGTTGCAGGA CCACTTCTGC GCTCGGCCCT TCCGGCTGGC 6000

TGGTTTATTG CTGATAAATC TGGAGCCGGT GAGCGTGGGT CTCGCGGTAT 6050

CATTGCAGCA CTGGGGCCAG ATGGTAAGCC CTCCCGTATC GTAGTTATCT 6100

ACACGACGGG GAGTCAGGCA ACTATGGATG AACGAAATAG ACAGATCGCT 6150

GAGATAGGTG CCTCACTGAT TAAGCATTGG TAACTGTCAG ACCAAGTTTA 6200

CTCATATATA CTTTAGATTG ATTTAAAACT TCATTTTTAA TTTAAAAGGA 6250

TCTAGGTGAA GATCCTTTTT GATAATCTCA TGACCAAAAT CCCTTAACGT 6300

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TTCTTGAGAT CCTTTTTTTC TGCGCGTAAT CTGCTGCTTG CAAACAAAAA 6400

AACCACCGCT ACCAGCGGTG GTTTGTTTGC CGGATCAAGA GCTACCAACT 6450

CTTTTTCCGA AGGTAACTGG CTTCAGCAGA GCGCAGATAC CAAATACTGT 6500

CCTTCTAGTG TAGCCGTAGT TAGGCCACCA CTTCAAGAAC TCTGTAGCAC 6550

CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC TGCTGCCAGT 6600

GGCGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA 6650

TAAGGCGCAG CGGTCGGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT 6700

TGGAGCGAAC GACCTACACC GAACTGAGAT ACCTACAGCG TGAGCATTGA 6750

GAAAGCGCCA CGCTTCCCGA AGGGAGAAAG GCGGACAGGT ATCCGGTAAG 6800

CGGCAGGGTC GGAACAGGAG AGCGCACGAG GGAGCTTCCA GGGGGAAACG 6850

CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG ACTTGAGCGT 6900

CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG 6950

CAACGCGGCC TTTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA 7000

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TGGAAAGCGG GCAGTGAGCG CAACGCAATT AATGTGAGTT ACCTCACTCA 7250

TTAGGCACCC CAGGCTTTAC ACTTTATGCT TCCGGCTCGT ATGTTGTGTG 7300

GAATTGTGAG CGGATAACAA TTTCACACAG GAAACAGCTA TGACCATGAT 7350

TACGAATTAA 7360

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6889 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT 50

TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC 100

TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG 150

ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA 200

TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC 250

ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT 300

AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC 350

TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC 400

GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA 450

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AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC 550

AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT 600

TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT 650

CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA 700

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AGATGCTTTC AAGTTCTCTG CTCCCCTCCT AAAGCTATGC ATTTTTATAA 1400

GACCATGGGA CTTTTGCTGG CTTTAGACCC CCTTGGCTTC GTTAGAACGC 1450

GGCTACAATT AATACATAAC CTTATGTATC ATACACATAG ATTTAGGTGA 1500

CACTATAGAA TAACATCCAC TTTGCCTTTC TCTCCACAGG TGTCACTCCA 1550

GGTCAACTGC ACCTCGGTTC TATCGATTGA ATTCCCCGGC CATAGCTGTC 1600

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TCAAAGGCTT CTACCCCAGC GACATCGCCG TGGAGTGGGA GAGCAGCGGG 2900

CAGCCGGAGA ACAACTACAA CACCACGCCT CCCATGCTGG ACTCCGACGG 2950

CTCCTTCTTC CTCTACAGCA AGCTCACCGT GGACAAGAGC AGGTGGCAGC 3000

AGGGGAACAT CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCGC 3050

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GGGGATCCTC TAGAGTCGAC CTGCAGAAGC TTGGCCGCCA TGGCCCAACT 3150

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TTCACAAATA AAGCATTTTT TTCACTGCAT TCTAGTTGTG GTTTGTCCAA 3250

ACTCATCAAT GTATCTTATC ATGTCTGGAT CGATCGGGAA TTAATTCGGC 3300

GCAGCACCAT GGCCTGAAAT AACCTCTGAA AGAGGAACTT GGTTAGGTAC 3350

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ATCGCCCTGA TAGACGGTTT TTCGCCCTTT GACGTTGGAG TCCACGTTCT 4250

TTAATAGTGG ACTCTTGTTC CAAACTGGAA CAACACTCAA CCCTATCTCG 4300

GGCTATTCTT TTGATTTATA AGGGATTTTG CCGATTTCGG CCTATTGGTT 4350

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TATGAGTATT CAACATTTCC GTGTCGCCCT TATTCCCTTT TTTGCGGCAT 4900

TTTGCCTTCC TGTTTTTGCT CACCCAGAAA CGCTGGTGAA AGTAAAAGAT 4950

GCTGAAGATC AGTTGGGTGC ACGAGTGGGT TACATCGAAC TGGATCTCAA 5000

CAGCGGTAAG ATCCTTGAGA GTTTTCGCCC CGAAGAACGT TTTCCAATGA 5050

TGAGCACTTT TAAAGTTCTG CTATGTGGCG CGGTATTATC CCGTGATGAC 5100

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CTTTATGCTT CCGGCTCGTA TGTTGTGTGG AATTGTGAGC GGATAACAAT 6850

TTCACACAGG AAACAGCTAT GACCATGATT ACGAATTAA 6889

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6557 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGAGTCGATC GACAGCTGTG 50

GAATGTGTGT CAGTTAGGGT GTGGAAAGTC CCCAGGCTCC CCAGCAGGCA 100

GAAGTATGCA AAGCATGCAT CTCAATTAGT CAGCAACCAG GTGTGGAAAG 150

TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC ATCTCAATTA 200

GTCAGCAACC ATAGTCCCGC CCCTAACTCC GCCCATCCCG CCCCTAACTC 250

CGCCCAGTTC CGCCCATTCT CCGCCCCATG GCTGACTAAT TTTTTTTATT 300

TATGCAGAGG CCGAGGCCGC CTCGGCCTCT GAGCTATTCC AGAAGTAGTG 350

AGGAGGCTTT TTTGGAGGCC TAGGCTTTTG CAAAAAGCTA GCTTATCCGG 400

CCGGGAACGG TGCATTGGAA CGCGGATTCC CCGTGCCAAG AGTGACGTAA 450

GTACCGCCTA TAGAGCGATA AGAGGATTTT ATCCCCGCTG CCATCATGGT 500

TCGACCATTG AACTGCATCG TCGCCGTGTC CCAAAATATG GGGATTGGCA 550

AGAACGGAGA CCTACCCTGG CCTCCGCTCA GGAACGAGTT CAAGTACTTC 600

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TATGGGTAGG AAAACCTGGT TCTCCATTCC TGAGAAGAAT CGACCTTTAA 700

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GGAGCTCATT TTCTTGCCAA AAGTTTGGAT GATGCCTTAA GACTTATTGA 800

ACAACCGGAA TTGGCAAGTA AAGTAGACAT GGTTTGGATA GTCGGAGGCA 850

GTTCTGTTTA CCAGGAAGCC ATGAATCAAC CAGGCCACCT TAGACTCTTT 900

GTGACAAGGA TCATGCAGGA ATTTGAAAGT GACACGTTTT TCCCAGAAAT 950

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TTGGGCACCC AGACCTACAT CTGCAACGTG AATCACAAGC CCAGCAACAC 2000

CAAGGTGGAC AAGAAAGTTG AGCCCAAATC TTGTGACAAA ACTCACACAT 2050

GCCCACCGTG CCCAGCACCT GAACTCCTGG GGGGACCGTC AGTCTTCCTC 2100

TTCCCCCCAA AACCCAAGGA CACCCTCATG ATCTCCCGGA CCCCTGAGGT 2150

CACATGCGTG GTGGTGGACG TGAGCCACGA AGACCCTGAG GTCAAGTTCA 2200

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CAAGAACCAG GTCAGCCTGA CCTGCCTGGT CAAAGGCTTC TATCCCAGCG 2500

ACATCGCCGT GGAGTGGGAG AGCAATGGGC AGCCGGAGAA CAACTACAAG 2550

ACCACGCCTC CCGTGCTGGA CTCCGACGGC TCCTTCTTCC TCTACAGCAA 2600

GCTCACCGTG GACAAGAGCA GGTGGCAGCA GGGGAACGTC TTCTCATGCT 2650

CCGTGATGCA TGAGGCTCTG CACAACCACT ACACGCAGAA GAGCCTCTCC 2700

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AGGTGAAGAT CCTTTTTGAT AATCTCATGA CCAAAATCCC TTAACGTGAG 5500

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TCTAGTGTAG CCGTAGTTAG GCCACCACTT CAAGAACTCT GTAGCACCGC 5750

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AGCGCCACGC TTCCCGAAGG GAGAAAGGCG GACAGGTATC CGGTAAGCGG 6000

CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG GGAAACGCCT 6050

GGTATCTTTA TAGTCCTGTC GGGTTTCGCC ACCTCTGACT TGAGCGTCGA 6100

TTTTTGTGAT GCTCGTCAGG GGGGCGGAGC CTATGGAAAA ACGCCAGCAA 6150

CGCGGCCTTT TTACGGTTCC TGGCCTTTTG CTGGCCTTTT GCTCACATGT 6200

TCTTTCCTGC GTTATCCCCT GATTCTGTGG ATAACCGTAT TACCGCCTTT 6250

GAGTGAGCTG ATACCGCTCG CCGCAGCCGA ACGACCGAGC GCAGCGAGTC 6300

AGTGAGCGAG GAAGCGGAAG AGCGCCCAAT ACGCAAACCG CCTCTCCCCG 6350

CGCGTTGGCC GATTCATTAA TCCAGCTGGC ACGACAGGTT TCCCGACTGG 6400

AAAGCGGGCA GTGAGCGCAA CGCAATTAAT GTGAGTTACC TCACTCATTA 6450

GGCACCCCAG GCTTTACACT TTATGCTTCC GGCTCGTATG TTGTGTGGAA 6500

TTGTGAGCGG ATAACAATTT CACACAGGAA ACAGCTATGA CCATGATTAC 6550

GAATTAA 6557

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7305 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT 50

TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC 100

TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG 150

ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA 200

TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC 250

ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT 300

AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC 350

TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC 400

GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA 450

TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA 500

AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC 550

AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT 600

TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT 650

CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA 700

TTGGAACGCG GATTCCCCGT GCCAAGAGTG ACGTAAGTAC CGCCTATAGA 750

GTCTATAGGC CCACCCCCTT GGCTTCGTTA GAACGCGGCT ACAATTAATA 800

CATAACCTTA TGTATCATAC ACATACGATT TAGGTGACAC TATAGAATAA 850

CATCCACTTT GCCTTTCTCT CCACAGGTGT CCACTCCCAG GTCCAACTGC 900

ACCTCGGTTC TAAGCTTATC GATATGAAAA AGCCTGAACT CACCGCGACG 950

TCTGTCGAGA AGTTTCTGAT CGAAAAGTTC GACAGCGTCT CCGACCTGAT 1000

GCAGCTCTCG GAGGGCGAAG AATCTCGTGC TTTCAGCTTC GATGTAGGAG 1050

GGCGTGGATA TGTCCTGCGG GTAAATAGCT GCGCCGATGG TTTCTACAAA 1100

GATCGTTATG TTTATCGGCA CTTTGCATCG GCCGCGCTCC CGATTCCGGA 1150

AGTGCTTGAC ATTGGGGAAT TCAGCGAGAG CCTGACCTAT TGCATCTCCC 1200

GCCGTGCACA GGGTGTCACG TTGCAACACC TGCCTGAAAC CGAACTGCCC 1250

GCTGTTCTGC AGCCGGTCGC GGAGGCCATG GATGCGATCG CTGCGGCCGA 1300

TCTTAGCCAG ACGAGCGGGT TCGGCCCATT CGGACCGCAA GGAATCGGTC 1350

AATACACTAC ATGGCGTGAT TTCATATGCG CGATTGCTGA TCCCCATGTG 1400

TATCACTGGC AAACTGTGAT GGACGACACC GTCAGTGCGT CCGTCGCGCA 1450

GGCTCTCGAT GAGCTGATGC TTTGGGCCGA GGACTGCCCC GAAGTCCGGC 1500

ACCTCGTGCA CGCGGATTTC GGCTCCAACA ATGTCCTGAC GGACAATGGC 1550

CGCATAACAG CGGTCATTGA CTGGAGCGAG GCGATGTTCG GGGATTCCCA 1600

ATACGAGGTC GCCAACATCT TCTTCTGGAG GCCGTGGTTG GCTTGTATGG 1650

AGCAGCAGAC GTACTTCGAG CGGAGGCATC CGGAGCTTGC AGGATCGCCG 1700

CGGCTCCGGG CGTATATGCT CCGCATTGGT CTTGACCAAC TCTATCAGAG 1750

CTTGGTTGAC GGCAATTTCG ATGATGCAGC TTGGGCGCAG GGTCGATGCG 1800

ACGCAATCGT CCGATCCGGA GCCGGGACTG TCGGGCGTAC ACAAATCGCC 1850

CGCAGAAGCG CGGCCGTCTG GACCGATGGC TGTGTAGAAG TACTCGCCGA 1900

TAGTGGAAAC CGACGCCCCA GCACTCGTCC GAGGGCAAAG GAATAGAGTA 1950

GATGCCGACC GAAGGATCCC CGGGGAATTC AATCGATGGC CGCCATGGCC 2000

CAACTTGTTT ATTGCAGCTT ATAATGGTTA CAAATAAAGC AATAGCATCA 2050

CAAATTTCAC AAATAAAGCA TTTTTTTCAC TGCATTCTAG TTGTGGTTTG 2100

TCCAAACTCA TCAATGTATC TTATCATGTC TGGATCGATC GGGAATTAAT 2150

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GGTACCTTCT GAGGCGGAAA GAACCAGCTG TGGAATGTGT GTCAGTTAGG 2250

GTGTGGAAAG TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC 2300

ATCTCAATTA GTCAGCAACC AGGTGTGGAA AGTCCCCAGG CTCCCCAGCA 2350

GGCAGAAGTA TGCAAAGCAT GCATCTCAAT TAGTCAGCAA CCATAGTCCC 2400

GCCCCTAACT CCGCCCATCC CGCCCCTAAC TCCGCCCAGT TCCGCCCATT 2450

CTCCGCCCCA TGGCTGACTA ATTTTTTTTA TTTATGCAGA GGCCGAGGCC 2500

GCCTCGGCCT CTGAGCTATT CCAGAAGTAG TGAGGAGGCT TTTTTGGAGG 2550

CCTAGGCTTT TGCAAAAAGC TAGCTTATCC GGCCGGGAAC GGTGCATTGG 2600

AACGCGGATT CCCCGTGCCA AGAGTCAGGT AAGTACCGCC TATAGAGTCT 2650

ATAGGCCCAC CCCCTTGGCT TCGTTAGAAC GCGGCTACAA TTAATACATA 2700

ACCTTTTGGA TCGATCCTAC TGACACTGAC ATCCACTTTT TCTTTTTCTC 2750

CACAGGTGTC CACTCCCAGG TCCAACTGCA CCTCGGTTCG CGAAGCTAGC 2800

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TTTCTAGTAG CAACTGCAAC TGGAGTACAT TCAGATATCC AGCTGACCCA 2900

GTCCCCGAGC TCCCTGTCCG CCTCTGTGGG CGATAGGGTC ACCATCACCT 2950

GCCGTGCCAG TCAGAGCGTC GATTACGATG GTGATAGCTA CATGAACTGG 3000

TATCAACAGA AACCAGGAAA AGCTCCGAAA CTACTGATTT ACGCGGCCTC 3050

GTACCTGGAG TCTGGAGTCC CTTCTCGCTT CTCTGGATCC GGTTCTGGGA 3100

CGGATTTCAC TCTGACCATC AGCAGTCTGC AGCCGGAAGA CTTCGCAACT 3150

TATTACTGTC AGCAAAGTCA CGAGGATCCG TACACATTTG GACAGGGTAC 3200

CAAGGTGGAG ATCAAACGAA CTGTGGCTGC ACCATCTGTC TTCATCTTCC 3250

CGCCATCTGA TGAGCAGTTG AAATCTGGAA CTGCCTCTGT TGTGTGCCTG 3300

CTGAATAACT TCTATCCCAG AGAGGCCAAA GTACAGTGGA AGGTGGATAA 3350

CGCCCTCCAA TCGGGTAACT CCCAGGAGAG TGTCACAGAG CAGGACAGCA 3400

AGGACAGCAC CTACAGCCTC AGCAGCACCC TGACGCTGAG CAAAGCAGAC 3450

TACGAGAAAC ACAAAGTCTA CGCCTGCGAA GTCACCCATC AGGGCCTGAG 3500

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GTTCACGTAG TGGGCCATCG CCCTGATAGA CGGTTTTTCG CCCTTTGACG 4650

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GATAAAGTTG CAGGACCACT TCTGCGCTCG GCCCTTCCGG CTGGCTGGTT 5950

TATTGCTGAT AAATCTGGAG CCGGTGAGCG TGGGTCTCGC GGTATCATTG 6000

CAGCACTGGG GCCAGATGGT AAGCCCTCCC GTATCGTAGT TATCTACACG 6050

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AGGTGCCTCA CTGATTAAGC ATTGGTAACT GTCAGACCAA GTTTACTCAT 6150

ATATACTTTA GATTGATTTA AAACTTCATT TTTAATTTAA AAGGATCTAG 6200

GTGAAGATCC TTTTTGATAA TCTCATGACC AAAATCCCTT AACGTGAGTT 6250

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CGCAGCGGTC GGGCTGAACG GGGGGTTCGT GCACACAGCC CAGCTTGGAG 6650

CGAACGACCT ACACCGAACT GAGATACCTA CAGCGTGAGC ATTGAGAAAG 6700

CGCCACGCTT CCCGAAGGGA GAAAGGCGGA CAGGTATCCG GTAAGCGGCA 6750

GGGTCGGAAC AGGAGAGCGC ACGAGGGAGC TTCCAGGGGG AAACGCCTGG 6800

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TTTGTGATGC TCGTCAGGGG GGCGGAGCCT ATGGAAAAAC GCCAGCAACG 6900

CGGCCTTTTT ACGGTTCCTG GCCTTTTGCT GGCCTTTTGC TCACATGTTC 6950

TTTCCTGCGT TATCCCCTGA TTCTGTGGAT AACCGTATTA CCGCCTTTGA 7000

GTGAGCTGAT ACCGCTCGCC GCAGCCGAAC GACCGAGCGC AGCGAGTCAG 7050

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CGTTGGCCGA TTCATTAATC CAGCTGGCAC GACAGGTTTC CCGACTGGAA 7150

AGCGGGCAGT GAGCGCAACG CAATTAATGT GAGTTACCTC ACTCATTAGG 7200

CACCCCAGGC TTTACACTTT ATGCTTCCGG CTCGTATGTT GTGTGGAATT 7250

GTGAGCGGAT AACAATTTCA CACAGGAAAC AGCTATGACC ATGATTACGA 7300

ATTAA 7305

CLAIMS

1. A DNA construct comprising a transcriptional initiation site, a transcriptional termination site, a selectable gene, a product gene provided 3′ to the selectable gene, a transcriptional regulatory region regulating transcription of both the selectable gene and the product gene, the selectable gene being positioned within an intron having a splice donor site 5′ of the intron, which splice donor site regulates expression of the product gene using the transcriptional regulatory region.

2. The DNA construct of claim 1 wherein the splice donor site comprises an efficient splice donor sequence.

3. The DNA construct of claim 2 wherein the splice donor site comprises a consensus splice donor sequence.

4. The DNA construct of claim 2 wherein the splice donor site comprises the sequence GACGTAAGT.

5. The DNA construct of claim 1 wherein the selectable gene is an amplifiable gene.

6. The DNA construct of claim 5 wherein the amplifiable gene is DHFR.

7. The DNA construct of claim 1 wherein the transcriptional regulatory region comprises a promoter and an enhancer.

8. A vector comprising the DNA construct of claim 1.

9. The vector of claim 8 wherein the selectable gene of the DNA construct is an amplifiable gene.

10. The vector of claim 8 that is capable of replication in a eukaryotic host.

11. A eukaryotic host cell comprising the vector of claim 10.

12. A eukaryotic host cell comprising the DNA construct of claim 5.

13. The host cell of claim 11 wherein the vector is introduced into the host cell by electroporation.

14. A eukaryotic host cell comprising the DNA construct of claim 1 integrated into a chromosome of the host cell.

15. The host cell of claim 14 that is a mammalian cell.

16. A method for producing a product of interest comprising culturing the host cell of claim 11 so as to express the product gene and recovering the product from the host cell culture.

17. The method of claim 16 further comprising recovering the product from the culture medium.

18. The method of claim 16 wherein the selectable gene is an amplifiable gene and the splice donor site comprises an efficient splice donor sequence.

19. A method for producing a product of interest comprising culturing the host cell of claim 12 so as to express the product gene in a selective medium comprising an amplifying agent for sufficient time to allow amplification to occur, and recovering the product.

20. A method for producing eukaryotic cells having multiple copies of a product gene comprising transforming eukaryotic cells with the DNA construct of claim 5, growing the cells in a selective medium comprising an amplifying agent for a sufficient time for amplification to occur, and selecting cells having multiple copies of the product gene.

21. The method of claim 20 further comprising recovering from the selected cells the product of interest.

FIG. 1A

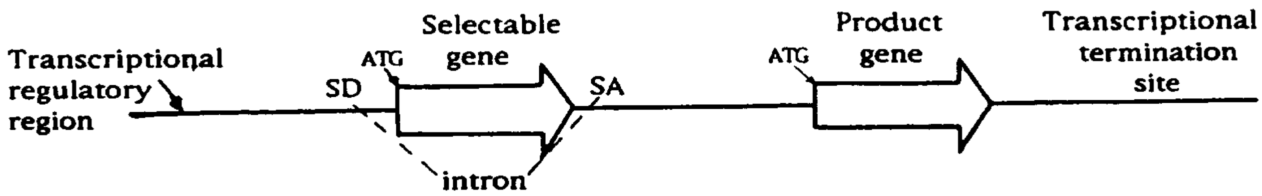


FIG. 1B

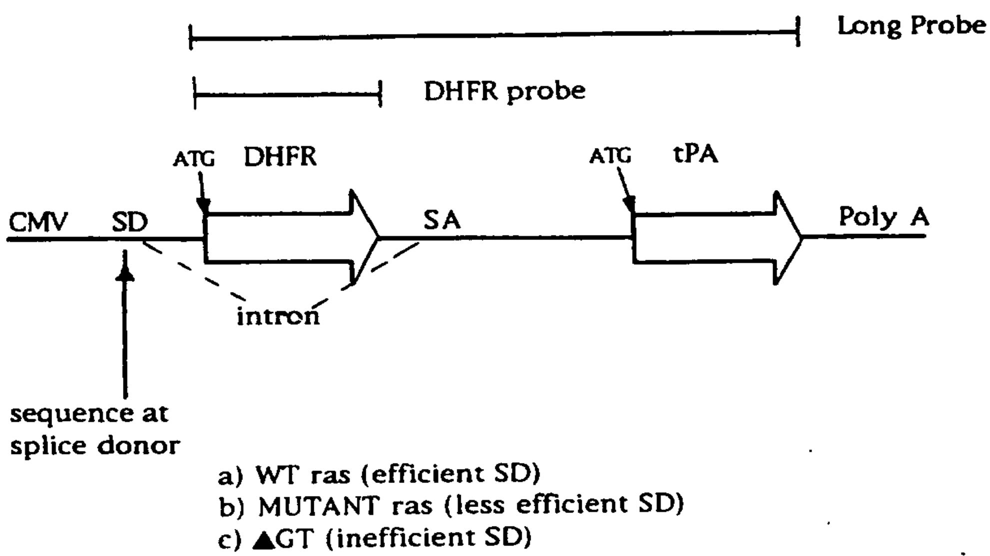


FIG. 1C

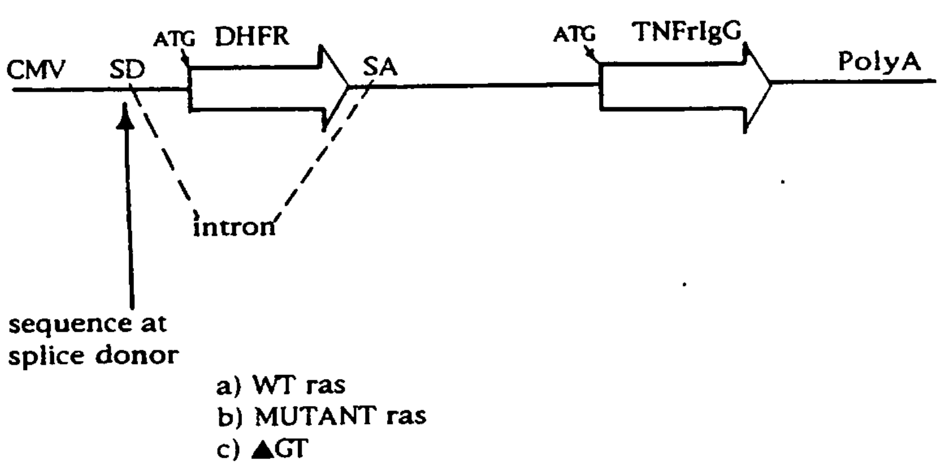


FIG. 1D

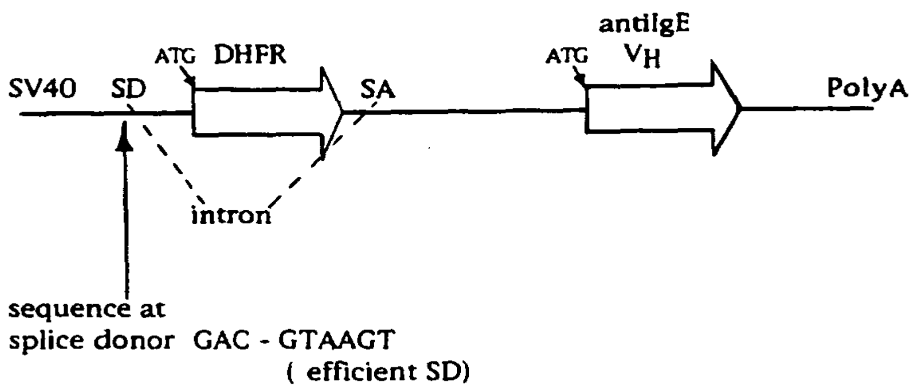

antiIgE
ATG
V_H
ATG
DHFR
SV40
SD
SA
PolyA
intron
sequence at
splice donor GAC - GTAAGT
(efficient SD)

FIG. 2

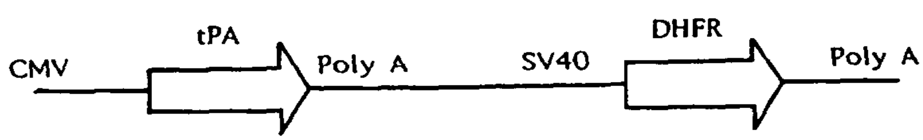

tPA
DHFR
CMV
Poly A
SV40
Poly A

FIG. 3A

```
          aluI
         sstI
         sacI
         hgiJII
         hgiAI/aspHI
         ecl136II
         bsp1286                                                                                     thaI
         bsiHKAI                                                                                     fnuDII/mvnI
         bmyI                       rmaI    tru9I                                                    bstUI
         banII                      maeI    mseI                                                     bsh1236I
       taqI                        speI    aseI/asnI/vspI                           bslI            aciI maeIII
    1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC
      AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCAATAATTA TCATTAGTTA ATGCCCCAGT AATCAAGTAT CGGGTATATA CCTCAAGGCG CAATGTATTG

                        scrFI
                        mvaI
                        ecoRII
                        dsaV
                     aciI
                   bglI bstNI                                   maeII
                  sau96I                                      hinlI/acyI
                  haeIII/palI     aciI                        ahaII/bsaHI
                  asuI  apyI[dcm+]              aciI     aatII          maeII           maeIII
  101 TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA
      AATGCCATTT ACCGGGCGGA CCGACTGGCG GGTTGCTGGG GGCGGGTAAC TGCAGTTATT ACTGCATACA AGGGTATCAT TGCGGTTATC CCTGAAAGGT

         maeII                                                                                        maeII
        hinlI/acyI                                                                                   hinlI/acyI
        ahaII/bsaHI                                     rsaI                        rsaI           ahaII/bsaHI
        aatII                              bglI         csp6I            ndeI       csp6I          aatII
  201 TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT
      AACTGCAGTT ACCCACCTCA TAAATGCCAT TTGACGGGTG AACCGTCATG TAGTTCACAT AGTATACGGT TCATGCGGGG GATAACTGCA GTTACTGCCA

                 scrFI
                 mvaI
                 ecoRII
             aciI                                                                                      nlaIII
           bglI  dsaV                                                                           styI
          sau96I bstNI            rsaI                                         maeII                  ncoI
          haeIII/palI             csp6I                               rsaI    snaBI                   dsaI hphI  aciI
          asuI   apyI[dcm+]    bsrI  nlaIII                           csp6I   bsaAI                   bsaJI   sfaNI
  301 AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC
      TTTACCGGGC GGACCGTAAT ACGGGTCATG TACTGGAATA CCCTGAAAGG ATGAACCGTC ATGTAGATGC ATAATCAGTA GCGATAATGG TACCACTACG
```

FIG. 3B

```
                                                                                   maeII
                                                                                  hinlI/acyI              nlaIV
               rsaI                          pleI                                 ahaII/bsaHI             hgiCI
               csp6I                  aciI   hinfI                bsmAI           aatII                   banI
401 GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA
    CCAAAACCGT CATGTAGTTA CCCGCACCTA TCGCCAAACT GAGTGCCCCT AAAGGTTCAG AGGTGGGGTA ACTGCAGTTA CCCTCAAACA AAACCGTGGT

                                                                                                          aluI
                                                                                                         sstI
                                                                                                         sacI
                                                                                                         hgiJII
                                                                                                         hgiAI/aspHI
                                                                                                         ecl136II
                                                                                                         bsp1286
                                                                                                         bsiHKAI
                                                                             rsaI                        bmyI
                                maeIII    aciI       hgaI        aciI        csp6I     mnlI              banII
501 AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT
    TTTAGTTGCC CTGAAAGGTT TTACAGCATT GTTGAGGCGG GGTAACTGCG TTTACCCGCC ATCCGCACAT GCCACCCTCC AGATATATTC GTCTCGAGCA

                                                                                                haeIII/palI
                                                                                               mcrI
                                                                                               eagI/xmaIII/eclXI
                                                                                               eaeI
                                                                                               cfrI
                                                                                              fnu4HI
                                                                                              aciI
                                                                                             thaI
                             esp3I                                                           fnuDII/mvnI
                        scrFI                                        sau96I           sacII/sstII
                        mvaI bsmAI                                         avaII            nspBII
                        ecoRII                                       asuI             kspI  scrFI
                        dsaV                                        nlaIV             dsaI  nciI
                        bstNI  hinlI/acyI                               scrFI          bglI bslI  mspI
                        apyI[dcm+]                                      nciI     sau3AI mnlI bstUI
                   sau3AI gsuI/bpmI                                     mspI     mboI/ndeII[dam-] hpaII
                   mboI/ndeII[dam-]                             mboII   hpaII    dpnI[dam+] bsaJI dsaV
                   dpnI[dam+]  hgaI  fokI                       bpuAI   dsaV     dpnII[dam-] bsh1236I
                   dpnII[dam-] ahaII/bsaHI             mnlI     bbsI    cauII    alwI[dam-] aciI  cauII
601 TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA
    AATCACTTGG CAGTCTAGCG GACCTCTGCG GTAGGTGCGA CAAAACTGGA GGTATCTTCT GTGGCCCTGG CTAGGTCGGA GGCGCCGGCC CTTGCCACGT
```

FIG. 3C

```
                tfiI
            aciI
           thaI hinfI                                                               fnu4HI
           fnuDII/mvnI                         aciI                                 bbvI
           bstUI                            rsaI                                  nspBII           taqI
           bsh1236I                         csp6I   scfI           mnlI           aciI       nlaIII
 701 TTGGAACGCG GATTCCCCGT GCCAAGAGTG CTGTAAGTAC CGCCTATAGA GCGATAAGAG GATTTTATCC CCGCTGCCAT CATGGTTCGA CCATTGAACT
     AACCTTGCGC CTAAGGGGCA CGGTTCTCAC GACATTCATG GCGGATATCT CGCTATTCTC CTAAAATAGG GGCGACGGTA GTACCAAGCT GGTAACTTGA

                                                                                  thaI
                                                                                  fnuDII/mvnI
                                                                                  bstUI
                                                                                  bsh1236I
                                                                                mluI
                                                                     bsrBI      aflIII     rsaI
                      pflMI                        bsmAI             aciI     xmnI         csp6I
     sfaNI            bslI                         bsaI           mnlI   ddeI asp700      scaI
 801 GCATCGTCGC CGTGTCCCAA AATATGGGGA TTGGCAAGAA CGGAGACCTA CCCTGCCCTC CGCTCAGGAA CGCGTTCAAG TACTTCCAAA GAATGACCAC
     CGTAGCAGCG GCACAGGGTT TTATACCCCT AACCGTTCTT GCCTCTGGAT GGGACGGGAG GCGAGTCCTT GCGCAAGTTC ATGAAGGTTT CTTACTGGTG

                                                            scrFI
                                                            mvaI
                                                            ecoRII
          eco57I                                            dsaV
         mboII                 tfiI                         bstNI                   tfiI       tru9I         tru9I
        earI/ksp632I           hinfI                        apyI[dcm+]          mboII  taqI    mseI         mseI
       mnlI                  alwNI    hphI                sexAI             ddeI    hinfI     ahaIII/draI   aseI/asnI/vspI
 901 AACCTCTTCA GTGGAAGGTA AACAGAATCT GGTGATTATG GGTAGGAAAA CCTGGTTCTC CATTCCTGAG AAGAATCGAC CTTTAAAGGA CAGAATTAAT
     TTGGAGAAGT CACCTTCCAT TTGTCTTAGA CCACTAATAC CCATCCTTTT GGACCAAGAG GTAAGGACTC TTCTTAGCTG GAAATTTCCT GTCTTAATTA

                                              aluI
                                             sstI
                                             sacI
                                             hgiJII
                                             hgiAI/aspHI
                                             ecl136II
                                             bsp1286
                                             bsiHKAI
                                             bmyI                                  tru9I               mspI
                                             banII                      sfaNI  mseI                hpaII
           ddeI                   bslI    mnlI                bstXI      fokI     aflII/bfrI         bsaWI
1001 ATAGTTCTCA GTAGAGAACT CAAAGAACCA CCACGAGGAG CTCATTTTCT TGCCAAAAGT TTGGATGATG CCTTAAGACT TATTGAACAA CCGGAATTGG
     TATCAAGAGT CATCTCTTGA GTTTCTTGGT GGTGCTCCTC GAGTAAAAGA ACGGTTTTCA AACCTACTAC GGAATTCTGA ATAACTTGTT GGCCTTAACC
```

FIG. 3D

```
                                                                               haeIII/palI
                                                                              haeI
                                                       scrFI          scrFI                        nlaIII
                                                       mvaI                 mvaI                            sau3AI
                                                       ecoRII         ecoRII                   mboI/ndeII[dam-]
                                                       dsaV   tfiI    dsaV    pleI             dpnI[dam+]
                                                       bstNI     nlaIII     bstNI     ddeI                  dpnII[dam-]
             accI  nlaIII             mnlI             apyI[dcm+]   hinfI   apyI[dcm+]    hinfI    maeIII  alwI[dam-]
1101 CAAGTAAAGT AGACATGGTT TGGATAGTCG GAGGCAGTTC TGTTTACCAG GAAGCCATGA ATCAACCAGG CCACCTTAGA CTCTTTGTGA CAAGGATCAT
     GTTCATTTCA TCTGTACCAA ACCTATCAGC CTCCGTCAAG ACAAATGGTC CTTCGGTACT TAGTTGGTCC GGTGGAATCT GAGAAACACT GTTCCTAGTA

                                                                                          mnlI
                                                                                     hinlI/acyI         scrFI
                                                                                     ahaII/bsaHI        mvaI
                                                                                  scrFI           ecoRII
                                                                                  mvaI    ecoNI         dsaV
                                                                                  ecoRII      sau96I
                                                                                  dsaV        avaII
                         maeII                                                    bstNI   bslI      asuI    mnlI
                       aflIII                                                     apyI[dcm+]      mnlI  bstNI
         apoI       maeIII                                       mnlI           bsaJI hgaI      ddeI    apyI[dcm+]
1201 GCAGGAATTT GAAAGTGACA CGTTTTTCCC AGAAATTGAT TTGGGGAAAT ATAAACCTCT CCCAGAATAC CCAGGCGTCC TCTCTGAGGT CCAGGAGGAA
     CGTCCTTAAA CTTTCACTGT GCAAAAAGGG TCTTTAACTA AACCCCTTTA TATTTGGAGA GGGTCTTATG GGTCCGCAGG AGAGACTCCA GGTCCTCCTT

                                                                                                   ppu10I
                                                             sfaNI                                 nsiI/avaIII
         sfaNI                   accI     mboII            mboII                        mnlI     aluI
1301 AAAGGCATCA AGTATAAGTT TGAAGTCTAC GAGAAGAAAG ACTAACAGGA AGATGCTTTC AAGTTCTCTG CTCCCCTCCT AAAGCTATGC ATTTTTATAA
     TTTCCGTAGT TCATATTCAA ACTTCAGATG CTCTTCTTTC TGATTGTCCT TCTACGAAAG TTCAAGAGAC GAGGGGAGGA TTTCGATACG TAAAAATATT

                                                         fnu4HI
        nlaIII                                           aciI
       styI                                             thaI
       ncoI                                             fnuDII/mvnI tru9I
       dsaI                       styI                  bstUI       mseI                                      maeIII
       bsaJI                      bsaJI                 bsh1236I   aseI/asnI/vspI                            hphI
1401 GACCATGGGA CTTTTGCTGG CTTTAGACCC CCTTGGCTTC GTTAGAACGC GGCTACAATT AATACATAAC CTTATGTATC ATACACATAG ATTTAGGTGA
     CTGGTACCCT GAAAACGACC GAAATCTGGG GGAACCGAAG CAATCTTGCG CCGATGTTAA TTATGTATTG GAATACATAG TATGTGTATC TAAATCCACT
```

FIG. 3E

```
                                             scrFI
                                             mvaI
                                             ecoRII                         bspMI
                                             dsaV                         scfI
                                             bstNI                            pstI
                                             apyI[dcm+]      mnlI       aluI   bsgI              tru9I
                                           gsuI/bpmI         bsaJI     hindIII fnu4HI            mseI
   scfI        fokI                     maeIII     hincII/hindII    ddeI       bbvI          apoI
1501 CACTATAGAA TAACATCCAC TTTGCCTTTC TCTCCACAGG TGTCACTCCA GGTCAACTGC ACCTCGGTTC TAAGCTTGGG CTGCAGGTCG CCGTGAATTT
     GTGATATCTT ATTGTAGGTG AAACGGAAAG AGAGGTGTCC ACAGTGAGGT CCAGTTGACG TGGAGCCAAG ATTCGAACCC GACGTCCAGC GGCACTTAAA

                                       hgiJII                                                 scrFI
                                       bsp1286                                                mvaI
                                       bmyI            fnu4HI                                 ecoRII
                                     mnlI              bbvI                   mboII           dsaV
                          sfaNI                                                                bstNI
                          fokI     mboII  banII      fnu4HI                 bpuAI              bstNI
     hgaI               nlaIII     earI/ksp632I      bbvI                   bbsI              apyI[dcm+] nlaIII
1601 AAGGGACGCT GTGAAGCAAT CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT TTCGCCCAGC CAGGAAATCC
     TTCCCTGCGA CACTTCGTTA GTACCTACGT TACTTCTCTC CCGAGACGAC ACACGACGAC GACACACCTC GTCAGAAGCA AAGCGGGTCG GTCCTTTAGG

                                          scfI
                         sau3AI           pstI
                         mboI/ndeII[dam-] bsgI                                                  hgiAI/aspHI
               nlaIV     dpnI[dam+]     sau3AI                                           hinPI  bsp1286
             mnlI        bstYI/xhoII    mboI/ndeII[dam-]                               fnu4HI   bsiHKAI
     tfiI   mboII        bglII          dpnI[dam+]                                     bbvI     bmyI
     hinfI  earI/ksp632I dpnII[dam-]    dpnII[dam-]                                 nlaIII  hhaI/cfoI
1701 ATGCCCGATT CAGAAGAGGA GCCAGATCTT ACCAAGTGAT CTGCAGAGAT GAAAAAACGC AGATGATATA CCAGCAACAT CAGTCATGGC TGCGCCCTGT
     TACGGGCTAA GTCTTCTCCT CGGTCTAGAA TGGTTCACTA GACGTCTCTA CTTTTTTGCG TCTACTATAT GGTCGTTGTA GTCAGTACCG ACGCGGGACA

            scrFI
            nciI
            mspI
            hpaII                                         alwNI
            dsaV                           bsp1286       ddeI                      fnu4HI  styI
   ddeI     cauII      sspI                bmyI         draIII                     bbvI    bsaJI
1801 GCTCAGAAGC AACCGGGTGG AATATTGCTG GTGCAACAGT GGCAGGGCAC AGTGCCACTC AGTGCCTGTC AAAAGTTGCA GCGAGCCAAG GTGTTTCAAC
     CGAGTCTTCG TTGGCCCACC TTATAACGAC CACGTTGTCA CCGTCCCGTG TCACGGTGAG TCACGGACAG TTTTCAACGT CGCTCGGTTC CACAAAGTTG
```

FIG. 3F

```
                                                                                                    pmlI
                                                                                                    eco72I
                                                                                                sau96I
                                                                                                asuI maeII
                                                                                            scrFI
          bspMI                                                                             mvaI haeIII/palI
       nlaIV         sau96I                                                                 ecoRII
       hgiCI         haeIII/palI                                                            dsaV
       banI          asuI  rsaI                    bsp1286                                  bstNI   bsaAI
      bsp1286       eco0109I/draII                 bmyI                                     bsaJI   bbrPI
      bmyI         alwNI   csp6I ddeI           bsrI     bcgI                               apyI[dcm+]
1901 GGGGGCACCT GCCAGCAGGC CCTGTACTTC TCAGATTTCG TGTGCCAGTG CCCCGAAGGA TTTGCTGGGA AGTGCTGTGA AATAGATACC AGGGCCACGT
     CCCCCGTGGA CGGTCGTCCG GGACATGAAG AGTCTAAAGC ACACGGTCAC GGGGCTTCCT AAACGACCCT TCACGACACT TTATCTATGG TCCCGGTGCA

               scrFI                                 hinPI
               mvaI                                       hhaI/cfoI
               ecoRII                               nlaIV   hgiAI/aspHI
               dsaV                                 narI    bsp1286              thaI
               bstNI                pmlI hgiAI/aspHI      kasI    bsiHKAI              fnuDII/mvnI
               bsaJI                eco72I                hinlI/acyI                   bstUI sau96I
           sau96I            bsaAI bsp1286          hgiCI   bmyI                 hinPI  haeIII/palI
           avaII             bbrPI bsiHKAI          haeII   apaLI/snoI           hhaI/cfoI
           asuI    sfaNI  scfI  bsp1286  bmyI   aciI      banI    alw44I/snoI     fnu4HI    asuI       bslI
         mnlI  apyI[dcm+] aluI  bmyI maeII     nspBII     ahaII/bsaHI    bsrI     bbvI bsh1236I      bslI
2001 GCTACGAGGA CCAGGGCATC AGCTACAGGG GCACGTGGAG CACAGCGGAG AGTGGCGCCG AGTGCACCAA CTGGAACAGC AGCGCGTTGG CCCAGAAGCC
     CGATGCTCCT GGTCCCGTAG TCGATGTCCC CGTGCACCTC GTGTCGCCTC TCACCGCGGC TCACGTGGTT GACCTTGTCG TCGCGCAACC GGGTCTTCGG

                                        scrFI
                                        mvaI
                                        ecoRII
                                        dsaV                            pleI      scrFI
                                        bstNI                               bsmAI       mvaI
                                        bsaJI                            taqI[dam-]     ecoRII
                       hinlI/acyI       apyI[dcm+]                     sau3AI hinfI     dsaV
             aciI haeIII/palI         haeIII/palI         scfI         mboI/ndeII[dam-] bstNI
       nspBII    haeI  hgaI bstXI    sau96I          pstI         dpnI[dam+]      apyI[dcm+]     tru9I
     scfI aciI  mnlI   ahaII/bsaHI   asuI     nlaIV       bsgI         dpnII[dam-]      bsaJI    maeII mseI
2101 CTACAGCGGG CGGAGGCCAG ACGCCATCAG GCTGGGCCTG GGGAACCACA ACTACTGCAG AAACCCAGAT CGAGACTCAA AGCCCTGGTG CTACGTCTTT
     GATGTCGCCC GCCTCCGGTC TGCGGTAGTC CGACCCGGAC CCCTTGGTGT TGATGACGTC TTTGGGTCTA GCTCTGAGTT TCGGGACCAC GATGCAGAAA
```

FIG. 3G

```
                            fnu4HI
                            bbvI
              aluI        scfI
            rsaI          pstI                  mnlI        maeIII                       dsaI
       aciI csp6I ddeI    bsgI                ddeI        alwNI                          bsaJI
2201 AAGGCGGGGA AGTACAGCTC AGAGTTCTGC AGCACCCCTG CCTGCTCTGA GGGAAACAGT GACTGCTACT TTGGGAATGG GTCAGCCTAC CGTGGCACGC
     TTCCGCCCCT TCATGTCGAG TCTCAAGACG TCGTGGGGAC GGACGAGACT CCCTTTGTCA CTGACGATGA AACCCTTACC CAGTCGGATG GCACCGTGCG
                                                                                            scrFI
                                                                                            pflMI
                                                                                            mvaI
                                                                                            ecoRII
                                                                                            dsaV
                                                      sau3AI                                bstNI
                                                      mboI/ndeII[dam-]                      bslI
                                                      dpnI[dam+]                            apyI[dcm+] haeIII/palI
             pleI  nlaIV                  ecoRI       dpnII[dam-]                        bsp1286       sau96I
        hphI hinfI hgiCI              dsaI apoI       alwI[dam-]                         bmyI alwNI    asuI
      mnlI  bcgI   banI mnlI   mnlI bsaJI          nlaIII                             bsrI  bsaJI   bsrI
2301 ACAGCCTCAC CGAGTCGGGT GCCTCCTGCC TCCCGTGGAA TTCCATGATC CTGATAGGCA AGGTTTACAC AGCACAGAAC CCCAGTGCCC AGGCACTGGG
     TGTCGGAGTG GCTCAGCCCA CGGAGGACGG AGGGCACCTT AAGGTACTAG GACTATCCGT TCCAAATGTG TCGTGTCTTG GGGTCACGGG TCCGTGACCC
                                                          nlaIV
                                                          hgiCI
                                                          banI  maeII
                                                       scrFI
     scrFI                                        mvaI    pmlI
     mvaI                                              ecoRII
     ecoRII                                       dsaV
     dsaV              tfiI                       bstNI    eco72I
     bstNI                  hinfI                 pflMI    bsaAI                               rsaI
     bsaJI                mspI                sfaNI    apyI[dcm+]      mboII                      csp6I      bsp1286
     apyI[dcm+]           hpaII              fokI bslI bsaJI      bbrPI eco57I  aciI      maeII  scaI       bmyI
2401 CCTGGGCAAA CATAATTACT GCCGGAATCC TGATGGGGAT GCCAAGCCCT GGTGCCACGT GCTGAAGAAC CGCAGGCTGA CGTGGGAGTA CTGTGATGTG
     GGACCCGTTT GTATTAATGA CGGCCTTAGG ACTACCCCTA CGGTTCGGGA CCACGGTGCA CGACTTCTTG GCGTCCGACT GCACCCTCAT GACACTACAC
                                                                                            scrFI
                                                                        mboII               mvaI
                                                                       earI/ksp632I         ecoRII
                                                                     hgiJII                 dsaV
                                                                     bsp1286                bstNI
                fnu4HI  bsmAI                                        bmyI                   apyI[dcm+]
                 aciI  ddeI    rsaI          ddeI                    banII               bslI bsaJI   fnu4HI
       mnlI   bspMI haeIII/palI csp6I       mnlI         sfaNI    mnlI sapI          mnlI    bslI     bbvI
2501 CCCTCCTGCT CCACCTGCGG CCTGAGACAG TACAGCCAGC CTCAGTTTCG CATCAAAGGA GGGCTCTTCG CCGACATCGC CTCCCACCCC TGGCAGGCTG
     GGGAGGACGA GGTGGACGCC GGACTCTGTC ATGTCGGTCG GAGTCAAAGC GTAGTTTCCT CCCGAGAAGC GGCTGTAGCG GAGGGTGGGG ACCGTCCGAC
```

FIG. 3H

```
                                  mspI
                                  hpaII                                                                        scrFI
                                  bslI                                                                         mvaI
                                 scrFI                                                                         ecoRII
                                 nciI       nlaIV                                                              dsaV
                                 dsaV     aciI                           aluI        tfiI        aciI          bstNI
              bslI       mnlI    cauII  bsrBI    bslI aciI              alwNI       hinfI      fnu4HI         apyI[dcm+]
2601 CCATCTTTGC CAAGCACAGG AGGTCGCCCG GAGAGCGGTT CCTGTGCGGG GGCATACTCA TCAGCTCCTG CTGGATTCTC TCTGCCGCCC ACTGCTTCCA
     GGTAGAAACG GTTCGTGTCC TCCAGCGGGC CTCTCGCCAA GGACACGCCC CCGTATGAGT AGTCGAGGAC GACCTAAGAG AGACGGCGGG TGACGAAGGT

                                                                    scrFI
                                                                    mvaI
                                                                    ecoRII
                                                                    dsaV
                                                                    bstNI
                                                                    apyI[dcm+]
                                                         scrFI     bslI
                                      sau3AI             nciI      bsaJI
                                      mboI/ndeII[dam-]   mspI   sau96I
                                      dpnI[dam+]         hpaII  nlaIV
                                      dpnII[dam-]        dsaV   avaII         mnlI
        mnlI    aciI    bslI      hphI[dam-]             cauII  asuI       mnlI         apoI       taqI
2701 GGAGAGGTTT CCGCCCCACC ACCTGACGGT GATCTTGGGC AGAACATACC GGGTGGTCCC TGGCGAGGAG GAGCAGAAAT TTGAAGTCGA AAAATACATT
     CCTCTCCAAA GGCGGGGTGG TGGACTGCCA CTAGAACCCG TCTTGTATGG CCCACCAGGG ACCGCTCCTC CTCGTCTTTA AACTTCAGCT TTTTATGTAA

                                                         scfI                               scrFI
                                                         pstI                               mvaI
                                                        fnu4HI                              ecoRII
                                                        bbvI pvuII                          dsaV
                                                     fnu4HI fnu4HI                          bstNI             aciI
                                                     bbvI   bbvI                       bsp1286      fnu4HI  sau96I
              ecoRI                                hinPI bsgI aluI        tfiI     nspBII  bsaJI     bbvI    avaII
              apoI taqI                            hhaI/cfoI nspBII       hinfI    aciI bmyI apyI[dcm+]      asuI
2801 GTCCATAAGG AATTCGATGA TGACACTTAC GACAATGACA TTGCGCTGCT GCAGCTGAAA TCGGATTCGT CCCGCTGTGC CCAGGAGAGC AGCGTGGTCC
     CAGGTATTCC TTAAGCTACT ACTGTGAATG CTGTTACTGT AACGCGACGA CGTCGACTTT AGCCTAAGCA GGGCGACACG GGTCCTCTCG TCGCACCAGG
```

FIG. 3I

```
                                                                  aluI
                                                                 sstI
                                       pvuII                     sacI
                 mspI      scfI                                  hgiJII
                 hpaII     pstI  fnu4HI                          hgiAI/aspHI
                scrFI    bspMI   bbvI                            ecl136II                haeIII/palI
                nciI    sau96I nspBII                            bsp1286                stuI
                dsaV    avaII fnu4HI     bsrI                    bsiHKAI                haeI
                cauII   asuI  bbvI    mspI                       bmyI    mspI          mnlI
     draIII   bslI bsaJI aciI  bsgI aluI hpaII                   banII   hpaII      nlaIII     bsmAI
2901 GCACTGTGTG CCTTCCCCCG GCGGACCTGC AGCTGCCGGA CTGGACGGAG TGTGAGCTCT CCGGCTACGG CAAGCATGAG GCCTTGTCTC CTTTCTATTC
     CGTGACACAC GGAAGGGGGC CGCCTGGACG TCGACGGCCT GACCTGCCTC ACACTCGAGA GGCCGATGCC GTTCGTACTC CGGAACAGAG GAAAGATAAG

                                                  fnu4HI
                                                 nspBII
      fnu4HI                                     aciI                                             nlaIII
      aciI                          rsaI fokI   fnu4HI                    tru9I       hphI       nspI
     bsrBI eco57I mnlI  nlaIII      csp6I    alwNI bbvI                   mseI       maeIII     nspHI      gsuI/bpmI
3001 GGAGCGGCTG AAGGAGGCTC ATGTCAGACT GTACCCATCC AGCCGCTGCA CATCACAACA TTTACTTAAC AGAACAGTCA CCGACAACAT GCTGTGTGCT
     CCTCGCCGAC TTCCTCCGAG TACAGTCTGA CATGGGTAGG TCGGCGACGT GTAGTGTTGT AAATGAATTG TCTTGTCAGT GGCTGTTGTA CGACACACGA

                 sau96I
                 nlaIV
                 haeIII/palI
                 asuI
                sau96I
                nlaIV scrFI                                    scrFI
                hgiJII                                              mvaI
                eco0109I/draII             scrFI               ecoRII
                bsp1286                    mvaI                     dsaV
                bsp120I                    ecoRII              bstNI
                bmyI  mvaI                 dsaV          sau96I
                banII ecoRII          bstNI              nlaIV                         aciI
              aciI    dsaV            bsaJI              haeIII/palI                  fnu4HI
           fnu4HI     bstNI         hinlI/acyI      tfiI    asuI apyI[dcm+]              haeIII/palI
           aciI asuI  apyI[dcm+]    hgaI       apyI[dcm+]  eco0109I/draII                eaeI
     bsmAI    bsrBI  apaI bsaJI     ahaII/bsaHI     hinfI   mnlI  bsaJI                  cfrI   nlaIII
3101 GGAGACACTC GGAGCGGCGG GCCCCAGGCA AACTTGCACG ACGCCTGCCA GGGCGATTCG GGAGGCCCCC TGGTGTGTCT GAACGATGGC CGCATGACTT
     CCTCTGTGAG CCTCGCCGCC CGGGGTCCGT TTGAACGTGC TGCGGACGGT CCCGCTAAGC CCTCCGGGGG ACCACACAGA CTTGCTACCG GCGTACTGAA
```

FIG. 3J

```
                                                        scrFI
                                                        nciI
                             scrFI                mspI
                             mvaI                       hpaII
                             ecoRII               dsaV
                             dsaV                 cauII
                             bstNI                     xmaI/pspAI
                             bsaJI                     smaI
                             apyI[dcm+]                scrFI
                        nlaIV                          nciI
                        eco0109I/draII           dsaV
                    aluI sau96I                  cauII     rsaI  styI maeIII                tfiI        nspI
                   pvuII asuI                          bsaJI     csp6I bsaJI            rmaI     hinfI       nspHI
           sfaNI   nspBII  haeIII/palI           fokI  avaI     bsp1407I   bstEII       maeI bsrI   maeIII nlaIII
3201 TGGTGGGCAT CATCAGCTGG GGCCTGGGCT GTGGACAGAA GGATGTCCCG GGTGTGTACA CCAAGGTTAC CAACTACCTA GACTGGATTC GTGACAACAT
     ACCACCCGTA GTAGTCGACC CCGGACCCGA CACCTGTCTT CCTACAGGGC CCACACATGT GGTTCCAATG GTTGATGGAT CTGACCTAAG CACTGTTGTA

                                                             mboII
                scrFI                            aciI earI/ksp632I
                mvaI                               sau3AI mnlI
                ecoRII                       mboI/ndeII[dam-]
                dsaV                         dpnI[dam+]            mboII
                bstNI            mnlI              dpnII[dam-]     eco57I
         bslI   apyI[dcm+]   pleI                  alwI[dam-]     mboII  bpuAI          hinPI
      mcrI maeIII            hinfI                bstYI/xhoII  mboII     bbsI           hhaI/cfoI      scfI
3301 GCGACCGTGA CCAGGAACAC CCGACTCCTC AAAAGCAAAT GAGATCCCGC CTCTTCTTCT TCAGAAGACA CTGCAAAGGC GCAGTGCTTC TCTACAGACT
     CGCTGGCACT GGTCCTTGTG GGCTGAGGAG TTTTCGTTTA CTCTAGGGCG GAGAAGAAGA AGTCTTCTGT GACGTTTCCG CGTCACGAAG AGATGTCTGA

                                        bsmAI               mboII
      gsuI/bpmI         aciI     aciI   bsaI  scfI     mnlI earI/ksp632I                                 tth111I/aspI
3401 TCTCCAGACC CACCACACCG CAGAAGCGGG ACGAGACCCT ACAGGAGAGG GAAGAGTGCA TTTTCCCAGA TACTTCCCAT TTTGGAAGTT TTCAGGACTT
     AGAGGTCTGG GTGGTGTGGC GTCTTCGCCC TGCTCTGGGA TGTCCTCTCC CTTCTCACGT AAAAGGGTCT ATGAAGGGTA AAACCTTCAA AAGTCCTGAA

                                                                                    scrFI
                                                                  scrFI       mvaI
                                                                  mvaI bsmI         ecoRII
                                                                  ecoRII      dsaV
                                                                  dsaV        bstNI
                                      mboII                       bstNI             bsaJI
                                      bpuAI   bsmI      rmaI    gsuI/bpmI   mnlI   bsaJI                    ecoRI   taqI
                                      bbsI nlaIII       maeI mnlI apyI[dcm+]   mnlI apyI[dcm+]             apoI   claI/bsp106
3501 GGTCTGATTT CAGGATACTC TGTCAGATGG GAAGACATGA ATGCACACTA GCCTCTCCAG GAATGCCTCC TCCCTGGGCA GAAGTGGGGG GAATTCAATC
     CCAGACTAAA GTCCTATGAG ACAGTCTACC CTTCTGTACT TACGTGTGAT CGGAGAGGTC CTTACGGAGG AGGGACCCGT CTTCACCCCC CTTAAGTTAG
```

FIG. 3K

```
        styI
      aciI
     fnu4HI   sau96I
     bglI nlaIII
    sfiI ncoI haeIII/palI
    haeIII/palI                   aluI
   eaeI  dsaI asuI             fnu4HI
   cfrI  bsaJI                 bbvI          maeIII         sfaNI  apoI                  bsmI
3601 GATGGCCGCC ATGGCCCAAC TTGTTTATTG CAGCTTATAA TGGTTACAAA TAAAGCAATA GCATCACAAA TTTCACAAAT AAAGCATTTT TTTCACTGCA
     CTACCGGCGG TACCGGGTTG AACAAATAAC GTCGAATATT ACCAATGTTT ATTTCGTTAT CGTAGTGTTT AAAGTGTTTA TTTCGTAAAA AAAGTGACGT

                                                            sau3AI
                                                            mboI/ndeII[dam-]
                                                            dpnI[dam+]
                                                            dpnII[dam-]
                                                           pvuI/bspCI
                                                           mcrI
                                                          taqI[dam-]  tru9I
                                                        claI/bsp106[dam-]          styI
                                                       sau3AI         mseI    fnu4HI    haeI
                                                       mboI/ndeII[dam-]       bbvI  ncoI
                                                       dpnI[dam+] xmnI     hinPI    dsaI haeIII/palI
   rmaI                                                dpnII[dam-]  aseI/asnI/vspI  bsaJI
   maeI                                         nlaIII alwI[dam-]  asp700  hhaI/cfoI nlaIII      mnlI
3701 TTCTAGTTGT GGTTTGTCCA AACTCATCAA TGTATCTTAT CATGTCTGGA TCGATCGGGA ATTAATTCGG CGCAGCACCA TGGCCTGAAA TAACCTCTGA
     AAGATCAACA CCAAACAGGT TTGAGTAGTT ACATAGAATA GTACAGACCT AGCTAGCCCT TAATTAAGCC GCGTCGTGGT ACCGGACTTT ATTGGAGACT

                  rsaI                                                                    nlaIV
                  csp6I                                                                 scrFI
                 nlaIV                                                                  mvaI
                 kpnI                                                                   ecoRII
                 hgiCI                                                                  dsaV
                 banI                      aluI                                         bstNI
                 asp718     mnlI          pvuII                                         apyI[dcm+]
   mnlI          acc65I   ddeI aciI       nspBII                                       bsaJI
3801 AAGAGGAACT TGGTTAGGTA CCTTCTGAGG CGGAAAGAAC CAGCTGTGGA ATGTGTGTCA GTTAGGGTGT GGAAAGTCCC CAGGCTCCCC AGCAGGCAGA
     TTCTCCTTGA ACCAATCCAT GGAAGACTCC GCCTTTCTTG GTCGACACCT TACACACAGT CAATCCCACA CCTTTCAGGG GTCCGAGGGG TCGTCCGTCT
```

FIG. 3L

```
                                                                nlaIV
                    sfaNI                 scrFI        scrFI                           sfaNI
                  ppu10I                  mvaI               mvaI                               ppu10I
                  nsiI/avaIII             ecoRII       ecoRII                        nsiI/avaIII
                 nlaIII                   dsaV         dsaV                         nlaIII
                sphI                      bstNI              bstNI                             sphI
                nspI                      apyI[dcm+]         apyI[dcm+]                        nspI
                nspHI                    sexAI              bsaJI                              nspHI
3901 AGTATGCAAA GCATGCATCT CAATTAGTCA GCAACCAGGT GTGGAAAGTC CCCAGGCTCC CCAGCAGGCA GAAGTATGCA AAGCATGCAT CTCAATTAGT
     TCATACGTTT CGTACGTAGA GTTAATCAGT CGTTGGTCCA CACCTTTCAG GGGTCCGAGG GGTCGTCCGT CTTCATACGT TTCGTACGTA GAGTTAATCA

                                                                                      nlaIII
                                                                                     styI
                                                                                     ncoI
                                           aciI                                bslI  dsaI
                    aciI         aciI  fokI             aciI bsrI  aciI        aciI  bsaJI
4001 CAGCAACCAT AGTCCCGCCC CTAACTCCGC CCATCCCGCC CCTAACTCCG CCCAGTTCCG CCCATTCTCC GCCCCATGGC TGACTAATTT TTTTTATTTA
     GTCGTTGGTA TCAGGGCGGG GATTGAGGCG GGTAGGGCGG GGATTGAGGC GGGTCAAGGC GGGTAAGAGG CGGGGTACCG ACTGATTAAA AAAAATAAAT

                                                                              rmaI
                                                                             styI
                    aciI                                                     bsaJI
                   fnu4HI                                                    blnI
                  sfiI                                                       avrII
                  haeIII/palI                                              haeIII/palI               tru9I
             bsaJI bglI          ddeI                                     stuI                      hpaI
           haeIII/palI mnlI haeIII/palI                     mnlI          haeI.                     hincII/hindII
         mnlI   mnlI   bsaJI  mnlI  aluI                mnlI             mnlI maeI              aluI mseI  aluI
4101 TGCAGAGGCC GAGGCCGCCT CGGCCTCTGA GCTATTCCAG AAGTAGTGAG GAGGCTTTTT TGGAGGCCTA GGCTTTTGCA AAAAGCTGTT AACAGCTTGG
     ACGTCTCCGG CTCCGGCGGA GCCGGAGACT CGATAAGGTC TTCATCACTC CTCCGAAAAA ACCTCCGGAT CCGAAAACGT TTTTCGACAA TTGTCGAACC

                                              scrFI
                                              mvaI
                                              ecoRII
         haeIII/palI                          dsaV
        eaeI                                  bstNI                                                aluI
        cfrI                      bsrI        apyI[dcm+]        tru9I       fnu4HI                pvuII
      bsrI               maeII maeIII        bsaJI   maeIII     mseI        bbvI   fokI           nspBII
4201 CACTGGCCGT CGTTTTACAA CGTCGTGACT GGGAAAACCC TGGCGTTACC CAACTTAATC GCCTTGCAGC ACATCCCCCC TTCGCCAGCT GGCGTAATAG
     GTGACCGGCA GCAAAATGTT GCAGCACTGA CCCTTTTGGG ACCGCAATGG GTTGAATTAG CGGAACGTCG TGTAGGGGGG AAGCGGTCGA CCGCATTATC
```

FIG. 3M

```
                                                                   hinPI
                                                                   hhaI/cfoI
                                                                  nlaIV
                      sau3AI                                      narI
           sau96I     mboI/ndeII[dam-]                            kasI
           haeIII/palI                                            hinlI/acyI
           asuI       dpnI[dam+]                                  hgiCI
         mnlI         dpnII[dam-]                                 haeII      aciI
      mboII   aciI   pvuI/bspCI                                   banI    sfaNI
      earI/ksp632I   mcrI                         bglI            ahaII/bsaHI                   sfaNI    aciI
4301 CGAAGAGGCC CGCACCGATC GCCCTTCCCA ACAGTTGCGT AGCCTGAATG GCGAATGGCG CCTGATGCGG TATTTTCTCC TTACGCATCT GTGCGGTATT
     GCTTCTCCGG GCGTGGCTAG CGGGAAGGGT TGTCAACGCA TCGGACTTAC CGCTTACCGC GGACTACGCC ATAAAAGAGG AATGCGTAGA CACGCCATAA

                                                                     aciI
                                                                  fnu4HI                  fnu4HI
                                        hinPI                    thaI                   hinPI
                                       thaI                      fnuDII/mvnI            hhaI/cfoI
                                       fnuDII/mvnI               bstUI                 thaI
                                       bstUI scfI     hinPI     hinPI                  fnuDII/mvnI
                                       bsh1236I       hhaI/cfoI hhaI/cfoI              bstUI
                                   rsaI hhaI/cfoI  fnu4HI   tru9I aciI                 bsh1236I     aciI
          aciI   maeII             csp6I   bslI    aciI     mseI bsh1236I          maeIII bbvI  maeIII
4401 TCACACCGCA TACGTCAAAG CAACCATAGT ACGCGCCCTG TAGCGGCGCA TTAAGCGCGG CGGGTGTGGT GGTTACGCGC AGCGTGACCG CTACACTTGC
     AGTGTGGCGT ATGCAGTTTC GTTGGTATCA TGCGCGGGAC ATCGCCGCGT AATTCGCGCC GCCCACACCA CCAATGCGCG TCGCACTGGC GATGTGAACG

                hinPI
                hhaI/cfoI                                                                          nlaIV
            rmaI                                                  mspI                            hgiJII
       hinPI haeII                                                hpaII                           bsp1286
       hhaI/cfoI    bsrBI                                        naeI                             bmyI
      haeII maeI    aciI            mboII                maeII cfr10I             aluI          banII        nlaIV
4501 CAGCGCCCTA GCGCCCGCTC CTTTCGCTTT CTTCCCTTCC TTTCTCGCCA CGTTCGCCGG CTTTCCCCGT CAAGCTCTAA ATCGGGGGCT CCCTTTAGGG
     GTCGCGGGAT CGCGGGCGAG GAAAGCGAAA GAAGGGAAGG AAAGAGCGGT GCAAGCGGCC GAAAGGGGCA GTTCGAGATT TAGCCCCCGA GGGAAATCCC

                         nlaIV                                       maeII    haeIII/palI
                         hgiCI taqI                                 draIII   sau96I
                         banI mnlI                       hphI       bsaAI    asuI
4601 TTCCGATTTA GTGCTTTACG GCACCTCGAC CCCAAAAAAC TTGATTTGGG TGATGGTTCA CGTAGTGGGC CATCGCCCTG ATAGACGGTT TTTCGCCCTT
     AAGGCTAAAT CACGAAATGC CGTGGAGCTG GGGTTTTTTG AACTAAACCC ACTACCAAGT GCATCACCCG GTAGCGGGAC TATCTGCCAA AAAGCGGGAA

       maeII pleI           tru9I     pleI                               bslI
      drdI   hinfI  maeII   mseI      hinfI          bsrI               bslI avaI
4701 TGACGTTGGA GTCCACGTTC TTTAATAGTG GACTCTTGTT CCAAACTGGA ACAACACTCA ACCCTATCTC GGGCTATTCT TTTGATTTAT AAGGGATTTT
     ACTGCAACCT CAGGTGCAAG AAATTATCAC CTGAGAACAA GGTTTGACCT TGTTGTGAGT TGGGATAGAG CCCGATAAGA AAACTAAATA TTCCCTAAAA
```

FIG. 3N

```
                                                                                                                      hgiAI/aspHI
                                                          thaI                                                        bsp1286
                                                          fnuDII/mvnI            maeII                                bsiHKAI
               tru9I                              tru9I   apoI tru9I            psp1406I                              bmyI   ddeI
               mseI                 tru9I         mseI bstUI   mseI           tru9I                                   apaLI/snoI
          haeIII/palI          aluI    mseI    apoI       bsh1236I         sspI mseI                                  alw44I/snoI
4801 GCCGATTTCG GCCTATTGGT TAAAAAATGA GCTGATTTAA CAAAAATTTA ACGCGAATTT TAACAAAATA TTAACGTTTA CAATTTTATG GTGCACTCTC
     CGGCTAAAGC CGGATAACCA ATTTTTTACT CGACTAAATT GTTTTTAAAT TGCGCTTAAA ATTGTTTTAT AATTGCAAAT GTTAAAATAC CACGTGAGAG

                                                                                                                             hinPI
                                                                                                                             hhaI/cfoI
                                                                                                                            thaI
                                                                               hinPI                                        fnuDII/mvnI
               aciI                                         maeIII          fnu4HI                                          bstUI
  rsaI        fnu4HI      tru9I                          maeII bsrI       nlaIII hhaI/cfoI                        nspBII bsh1236I
  csp6I     sfaNI         mseI        aciI              bsaAI tth111I/aspI bbvI                   aciI            aciI hgaI
4901 AGTACAATCT GCTCTGATGC CGCATAGTTA AGCCAACTCC GCTATCGCTA CGTGACTGGG TCATGGCTGC GCCCCGACAC CCGCCAACAC CCGCTGACGC
     TCATGTTAGA CGAGACTACG GCGTATCAAT TCGGTTGAGG CGATAGCGAT GCACTGACCC AGTACCGACG CGGGGCTGTG GGCGGTTGTG GGCGACTGCG

                                                                  scrFI
                           sfaNI                                  nciI
                         mspI                                     mspI
                         hpaII                                    hpaII       nspI
                        scrFI                                     dsaV        nspHI
                        nciI                                 esp3I         fnu4HI
                        dsaV fokI                        maeIII bsmAI      bbvI
        drdI            cauII   aciI           aluI        bslI  cauII aluI nlaIII    mnlI     hphI      hphI
5001 GCCCTGACGG GCTTGTCTGC TCCCGGCATC CGCTTACAGA CAAGCTGTGA CCGTCTCCGG GAGCTGCATG TGTCAGAGGT TTTCACCGTC ATCACCGAAA
     CGGGACTGCC CGAACAGACG AGGGCCGTAG GCGAATGTCT GTTCGACACT GGCAGAGGCC CTCGACGTAC ACAGTCTCCA AAAGTGGCAG TAGTGGCTTT

      thaI
      fnuDII/mvnI
      bstUI
      bsh1236I
     hinPI
     hhaI/cfoI                            mnlI
     thaI mnlI                          haeIII/palI                                                        hinlI/acyI
     fnuDII/mvnI          mboII        sau96I                                     nlaIII                   ahaII/bsaHI
     bstUI                bpuAI        asuI                                tru9I  rcaI                     aatII
     bsh1236I             bbsI        eco0109I/draII                       mseI   bspHI                ddeI maeII
5101 CGCGCGAGGC AGTATTCTTG AAGACGAAAG GGCCTCGTGA TACGCCTATT TTTATAGGTT AATGTCATGA TAATAATGGT TTCTTAGACG TCAGGTGGCA
     GCGCGCTCCG TCATAAGAAC TTCTGCTTTC CCGGAGCACT ATGCGGATAA AAATATCCAA TTACAGTACT ATTATTACCA AAGAATCTGC AGTCCACCGT
```

FIG. 30

```
                       nlaIV
                     aciI
                     thaI
                     fnuDII/mvnI
                     bstUI
                     bsh1236I
                    hinPI
                    hhaI/cfoI
                                                                                  rcaI
                                                                                  bspHI
                                                                              bsrBI    bsmAI
                                                                              aciI  nlaIII
5201 CTTTTCGGGG AAATGTGCGC GGAACCCCTA TTTGTTTATT TTTCTAAATA CATTCAAATA TGTATCCGCT CATGAGACAA TAACCCTGAT AAATGCTTCA
     GAAAAGCCCC TTTACACGCG CCTTGGGGAT AAACAAATAA AAAGATTTAT GTAAGTTTAT ACATAGGCGA GTACTCTGTT ATTGGGACTA TTTACGAAGT

                   mboII                                                   fnu4HI
       sspI        earI/ksp632I                                            aciI                         hphI
5301 ATAATATTGA AAAAGGAAGA GTATGAGTAT TCAACATTTC CGTGTCGCCC TTATTCCCTT TTTTGCGGCA TTTTGCCTTC CTGTTTTTGC TCACCCAGAA
     TATTATAACT TTTTCCTTCT CATACTCATA AGTTGTAAAG GCACAGCGGG AATAAGGGAA AAAACGCCGT AAAACGGAAG GACAAAAACG AGTGGGTCTT

                                         hgiAI/aspHI
                                         bsp1286
                               sau3AI    bsiHKAI                          sau3AI   nspBII    sau3AI
                               mboI/ndeII[dam-]                           mboI/ndeII[dam-]   mboI/ndeII[dam-]
                               dpnI[dam+] bmyI                            dpnI[dam+]         dpnI[dam+]
                               dpnII[dam-]                               bstYI/xhoII         dpnII[dam-]
                          eco57I         apaLI/snoI                   bsrI dpnII[dam-]       alwI[dam-]
         hphI          sfaNI  mboII[dam-] alw44I/snoI  maeIII taqI     alwI[dam-]  aciI   bstYI/xhoII
5401 ACGCTGGTGA AAGTAAAAGA TGCTGAAGAT CAGTTGGGTG CACGAGTGGG TTACATCGAA CTGGATCTCA ACAGCGGTAA GATCCTTGAG AGTTTTCGCC
     TGCGACCACT TTCATTTTCT ACGACTTCTA GTCAACCCAC GTGCTCACCC AATGTAGCTT GACCTAGAGT TGTCGCCATT CTAGGAACTC TCAAAAGCGG

                                                                                   scrFI
                                                        aciI                       nciI
                                                      thaI                         mspI
          maeII                                       fnuDII/mvnI                  hpaII
         psp1406I            hgiAI/aspHI              bstUI                        dsaV
         xmnI                bsp1286  tru9I           bsh1236I                 hinlI/acyI
         asp700              bsiHKAI  mseI           hinPI                     hgaI cauII
       mboII                 bmyI   ahaIII/draI      hhaI/cfoI                 ahaII/bsaHI     bcgI  mcrI  fnu4HI
5501 CCGAAGAACG TTTTCCAATG ATGAGCACTT TTAAAGTTCT GCTATGTGGC GCGGTATTAT CCCGTGATGA CGCCGGGCAA GAGCAACTCG GTCGCCGCAT
     GGCTTCTTGC AAAAGGTTAC TACTCGTGAA AATTTCAAGA CGATACACCG CGCCATAATA GGGCACTACT GCGGCCCGTT CTCGTTGAGC CAGCGGCGTA

                               rsaI
                               csp6I  bsrI                                                          fnu4HI
           ddeI               scaI  hphI  maeIII      sfaNI    fokI   nlaIII                        bbvI      nlaIII
5601 ACACTATTCT CAGAATGACT TGGTTGAGTA CTCACCAGTC ACAGAAAAGC ATCTTACGGA TGGCATGACA GTAAGAGAAT TATGCAGTGC TGCCATAACC
     TGTGATAAGA GTCTTACTGA ACCAACTCAT GAGTGGTCAG TGTCTTTTCG TAGAATGCCT ACCGTACTGT CATTCTCTTA ATACGTCACG ACGGTATTGG
```

FIG. 3P

```
                                                      sau96I
                                                      avaII
                                              sau3AI  asuI                                         nlaIII
                       haeIII/palI            mboI/ndeII[dam-]                                   sau3AI maeIII
                      eaeI                    dpnI[dam+]                                         mboI/ndeII[dam-]
                      cfrI                    dpnII[dam-]                                        dpnI[dam+]
                     fnu4HI                  pvuI/bspCI                                          dpnII[dam-]
                     aciI                    mcrI   mnlI         aluI   aciI             nlaIII alwI[dam-]
5701 ATGAGTGATA ACACTGCGGC CAACTTACTT CTGACAACGA TCGGAGGACC GAAGGAGCTA ACCGCTTTTT TGCACAACAT GGGGATCAT GTAACTCGCC
     TACTCACTAT TGTGACGCCG GTTGAATGAA GACTGTTGCT AGCCTCCTGG CTTCCTCGAT TGGCGAAAAA ACGTGTTGTA CCCCCTAGTA CATTGAGCGG

                                                                                                  hinPI
                    mspI                                                                          hhaI/cfoI
       sau3AI   nlaIV                                                                            mstI
       mboI/ndeII[dam-] aluI                                                                     aviII/fspI    bsrI
       dpnI[dam+]   hpaII                                                   fnu4HI           maeII         tru9I
       dpnII[dam-] bsaWI                                 maeIII     sfaNI   bbvI           psp1406I        mseI
5801 TTGATCGTTG GGAACCGGAG CTGAATGAAG CCATACCAAA CGACGAGCGT GACACCACGA TGCCAGCAGC AATGGCAACA ACGTTGCGCA AACTATTAAC
     AACTAGCAAC CCTTGGCCTC GACTTACTTC GGTATGGTTT GCTGCTCGCA CTGTGGTGCT ACGGTCGTCG TTACCGTTGT TGCAACGCGT TTGATAATTG

                               mspI
                               hpaII                                                              bglI
                              scrFI                                                              sau96I
                        aluI  nciI        tru9I      fokI                     sau96I             haeIII/palI
                      rmaI    dsaV        mseI    bsrI        aciI            avaII       hinPI  asuI   mspI
                      maeI    cauII      aseI/asnI/vspI   mnlI                asuI        hhaI/cfoI     hpaII
5901 TGGCGAACTA CTTACTCTAG CTTCCCGGCA ACAATTAATA GACTGGATGG AGGCGGATAA AGTTGCAGGA CCACTTCTGC GCTCGGCCCT TCCGGCTGGC
     ACCGCTTGAT GAATGAGATC GAAGGGCCGT TGTTAATTAT CTGACCTACC TCCGCCTATT TCAACGTCCT GGTGAAGACG CGAGCCGGGA AGGCCGACCG

                                mspI               thaI
                                hpaII              fnuDII/mvnI             haeIII/palI
                               cfr10I              bstUI                  sau96I
                            nlaIV hphI        bsmAI aciI        fnu4HI   nlaIV
                         gsuI/bpmI           bsaI  bsh1236I     bbvI bsrI asuI             mnlI
6001 TGGTTTATTG CTGATAAATC TGGAGCCGGT GAGCGTGGGT CTCGCGGTAT CATTGCAGCA CTGGGGCCAG ATGGTAAGCC CTCCCGTATC GTAGTTATCT
     ACCAAATAAC GACTATTTAG ACCTCGGCCA CTCGCACCCA GAGCGCCATA GTAACGTCGT GACCCCGGTC TACCATTCGG GAGGGCATAG CATCAATAGA

                                                         ddeI
                                                    sau3AI        nlaIV
                pleI                                mboI/ndeII[dam-]
                hinfI                               dpnI[dam+]    hgiCI         tru9I
         eam1105I               fokI                dpnII[dam-]   banI mnlI     mseI       maeIII
6101 ACACGACGGG GAGTCAGGCA ACTATGGATG AACGAAATAG ACAGATCGCT GAGATAGGTG CCTCACTGAT TAAGCATTGG TAACTGTCAG ACCAAGTTTA
     TGTGCTGCCC CTCAGTCCGT TGATACCTAC TTGCTTTATC TGTCTAGCGA CTCTATCCAC GGAGTGACTA ATTCGTAACC ATTGACAGTC TGGTTCAAAT
```

FIG. 3Q

```
                                                          rmaI       sau3AI
                                                      sau3AI hphI    mboI/ndeII[dam-]
                                                      mboI/ndeII[dam-]
                                                      dpnI[dam+]     dpnI[dam+]
                                                      dpnII[dam-]    dpnII[dam-]
                    tru9I                       tru9I bstYI/xhoII    alwI[dam-]          nlaIII                maeII
                    mseI                  tru9I mseI  alwI[dam-]   bstYI/xhoII          rcaI               tru9I
                   ahaIII/draI            mseI ahaIII/draI maeI  mboII[dam-]            bspHI              mseI
6201 CTCATATATA CTTTAGATTG ATTTAAAACT TCATTTTTAA TTTAAAAGGA TCTAGGTGAA GATCCTTTTT GATAATCTCA TGACCAAAAT CCCTTAACGT
     GAGTATATAT GAAATCTAAC TAAATTTTGA AGTAAAAATT AAATTTTCCT AGATCCACTT CTAGGAAAAA CTATTAGAGT ACTGGTTTTA GGGAATTGCA

                                                    sau3AI
                                                    mboI/ndeII[dam-]
                                                    dpnI[dam+]  sau3AI
                                                    dpnII[dam-] mboI/ndeII[dam-] thaI
                                                   bstYI/xhoII  dpnI[dam+]       fnuDII/mvnI
                                           sau3AI  alwI[dam-]   dpnII[dam-]      bstUI
                                           mboI/ndeII[dam-]     alwI[dam-]       bsh1236I
                 hgaI                      dpnI[dam+] mboII[dam-]               hinPI      fnu4HI
               ddeI                        dpnII[dam-]         bstYI/xhoII      hhaI/cfoI  bbvI
6301 GAGTTTTCGT TCCACTGAGC GTCAGACCCC GTAGAAAAGA TCAAAGGATC TTCTTGAGAT CCTTTTTTTC TGCGCGTAAT CTGCTGCTTG CAAACAAAAA
     CTCAAAAGCA AGGTGACTCG CAGTCTGGGG CATCTTTTCT AGTTTCCTAG AAGAACTCTA GGAAAAAAAG ACGCGCATTA GACGACGAAC GTTTGTTTTT

                                     sau3AI
                                     mboI/ndeII[dam-]
                                     dpnI[dam+]
                                     dpnII[dam-]
                                    alwI[dam-]
                 aciI             mspI                              bsrI             hinPI
       aciI    nspBII             hpaII      aluI                 maeIII    eco57I   hhaI/cfoI
6401 AACCACCGCT ACCAGCGGTG GTTTGTTTGC CGGATCAAGA GCTACCAACT CTTTTTCCGA AGGTAACTGG CTTCAGCAGA GCGCAGATAC CAAATACTGT
     TTGGTGGCGA TGGTCGCCAC CAAACAAACG GCCTAGTTCT CGATGGTTGA GAAAAAGGCT TCCATTGACC GAAGTCGTCT CGCGTCTATG GTTTATGACA

                                                                                                      fnu4HI
                                                                                               alwNI  bbvI
                                                                                              bsrI  fnu4HI
      rmaI                  haeIII/palI
      maeI        bslI     haeI                 scfI     aciI        mnlI               maeIII     bbvI     bsrI
6501 CCTTCTAGTG TAGCCGTAGT TAGGCCACCA CTTCAAGAAC TCTGTAGCAC CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC TGCTGCCAGT
     GGAAGATCAC ATCGGCATCA ATCCGGTGGT GAAGTTCTTG AGACATCGTG GCGGATGTAT GGAGCGAGAC GATTAGGACA ATGGTCACCG ACGACGGTCA
```

FIG. 3R

```
                         scrFI                                       aciI                       hgiAI/aspHI
                         nciI                                      nspBII                       bsp1286
                         mspI                         mspI        fnu4HI                        bsiHKAI
                         hpaII                        hpaII       bbvI                          bmyI
                         dsaV     pleI               bsaWI      hinPI  mcrI                     apaLI/snoI
                         cauII    hinfI           maeIII        hhaI/cfoI                       alw44I/snoI   aluI
6601 GGCGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA TAAGGCGCAG CGGTCGGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT
     CCGCTATTCA GCACAGAATG GCCCAACCTG AGTTCTGCTA TCAATGGCCT ATTCCGCGTC GCCAGCCCGA CTTGCCCCCC AAGCACGTGT GTCGGGTCGA

                                                                                                          mspI
                                                                hinPI                                     hpaII
                                                                hhaI/cfoI                                 bslI   fnu4HI
                              ddeI      scfI                   haeII                          aciI       bsaWI   aciI
6701 TGGAGCGAAC GACCTACACC GAACTGAGAT ACCTACAGCG TGAGCATTGA GAAAGCGCCA CGCTTCCCGA AGGGAGAAAG GCGGACAGGT ATCCGGTAAG
     ACCTCGCTTG CTGGATGTGG CTTGACTCTA TGGATGTCGC ACTCGTAACT CTTTCGCGGT GCGAAGGGCT TCCCTCTTTC CGCCTGTCCA TAGGCCATTC

                                             scrFI
                                             mvaI           scrFI
                                             ecoRII   mvaI
                                             dsaV     ecoRII
                                             bstNI          dsaV
                            hinPI mnlI       bsaJI          bstNI                                                taqI
                            hhaI/cfoI   aluI apyI[dcm+]     apyI[dcm+]                       mnlI drdI       hgaI
6801 CGGCAGGGTC GGAACAGGAG AGCGCACGAG GGAGCTTCCA GGGGGAAACG CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG ACTTGAGCGT
     GCCGTCCCAG CCTTGTCCTC TCGCGTGCTC CCTCGAAGGT CCCCCTTTGC GGACCATAGA AATATCAGGA CAGCCCAAAG CGGTGGAGAC TGAACTCGCA

                                                                                      haeIII/palI
                                                                  haeIII/palI      scrFI
                                                                fnu4HI             mvaI bslI
                                                                aciI               ecoRII
                                                               thaI bslI           dsaV                      nlaIII
                                                               fnuDII/mvnI         bstNI                         nspI
                                   nlaIV                       bstUI               apyI[dcm+]     haeIII/palI    nspHI
                sfaNI            aciI                          bsh1236I       nlaIV haeI          haeI           aflIII
6901 CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG CAACGCGGCC TTTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA
     GCTAAAAACA CTACGAGCAG TCCCCCCGCC TCGGATACCT TTTTGCGGTC GTTGCGCCGG AAAAATGCCA AGGACCGGAA AACGACCGGA AAACGAGTGT

                                                                                       fnu4HI             fnu4HI
                                                                                       bbvI               bbvI pleI
                              tfiI                                               bsrBI   aciI             hinPI  hinfI
                              hinfI                   aciI          aluI         aciI   fnu4HI      mcrI  hhaI/cfoI
7001 TGTTCTTTCC TGCGTTATCC CCTGATTCTG TGGATAACCG TATTACCGCC TTTGAGTGAG CTGATACCGC TCGCCGCAGC CGAACGACCG AGCGCAGCGA
     ACAAGAAAGG ACGCAATAGG GGACTAAGAC ACCTATTGGC ATAATGGCGG AAACTCACTC GACTATGGCG AGCGGCGTCG GCTTGCTGGC TCGCGTCGCT
```

FIG. 3S

```
                                                                   thaI
                                                                   fnuDII/mvnI
                                                                   bstUI
                                                                   bsh1236I
                                                                  hinPI
                                                                  hhaI/cfoI
                                                                 thaI
                                                                 fnuDII/mvnI
                       sapI  hinPI                               bstUI   haeIII/palI         aluI
                       mboII hhaI/cfoI                           bsh1236I          tru9I   pvuII
                       earI/ksp632I                 mnlI        bslI    eaeI   tfiI aseI/asnI/vspI
                 mnlI  aciI    haeII             aciI           aciI    cfrI   hinfI mseI    nspBII                  bsrI
7101 GTCAGTGAGC GAGGAAGCGG AAGAGCGCCC AATACGCAAA CCGCCTCTCC CCGCGCGTTG GCCGATTCAT TAATCCAGCT GGCACGACAG GTTTCCCGAC
     CAGTCACTCG CTCCTTCGCC TTCTCGCGGG TTATGCGTTT GGCGGAGAGG GGCGCGCAAC CGGCTAAGTA ATTAGGTCGA CCGTGCTGTC CAAAGGGCTG

                                                                        scrFI
                                                                        mvaI
                                                                        ecoRII
                                                                        dsaV
                                   tru9I                           nlaIV bstNI
                       hinPI       mseI       maeIII               hgiCI apyI[dcm+]                mspI
           aciI        hhaI/cfoI  aseI/asnI/vspI  mnlI             banI bsaJI                      hpaII
7201 TGGAAAGCGG GCAGTGAGCG CAACGCAATT AATGTGAGTT ACCTCACTCA TTAGGCACCC CAGGCTTTAC ACTTTATGCT TCCGGCTCGT ATGTTGTGTG
     ACCTTTCGCC CGTCACTCGC GTTGCGTTAA TTACACTCAA TGGAGTGAGT AATCCGTGGG GTCCGAAATG TGAAATACGA AGGCCGAGCA TACAACACAC

                                                                     tru9I
                                                                     mseI
                                                                    aseI/asnI/vspI
               aciI                                                xmnI
             bsrBI                          aluI      nlaIII       asp700
7301 GAATTGTGAG CGGATAACAA TTTCACACAG GAAACAGCTA TGACCATGAT TACGAATTAA
     CTTAACACTC GCCTATTGTT AAAGTGTGTC CTTTGTCGAT ACTGGTACTA ATGCTTAATT
```

>length: 7360

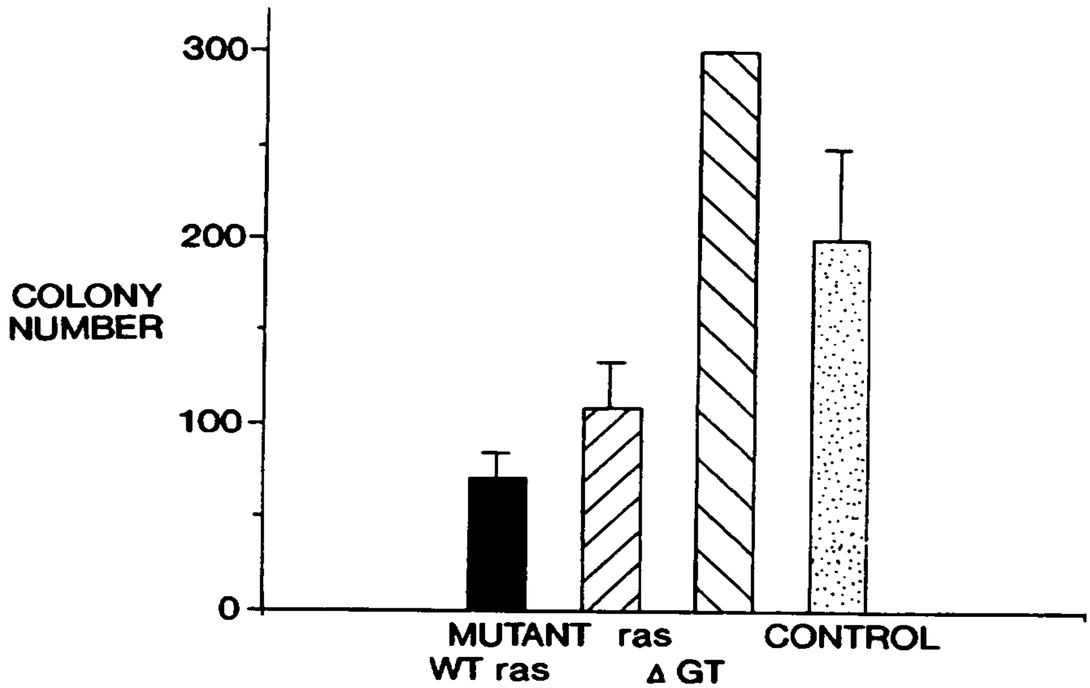

300
200
100
0
COLONY
NUMBER
MUTANT
WT ras
ras
Δ GT
CONTROL

FIG. 4

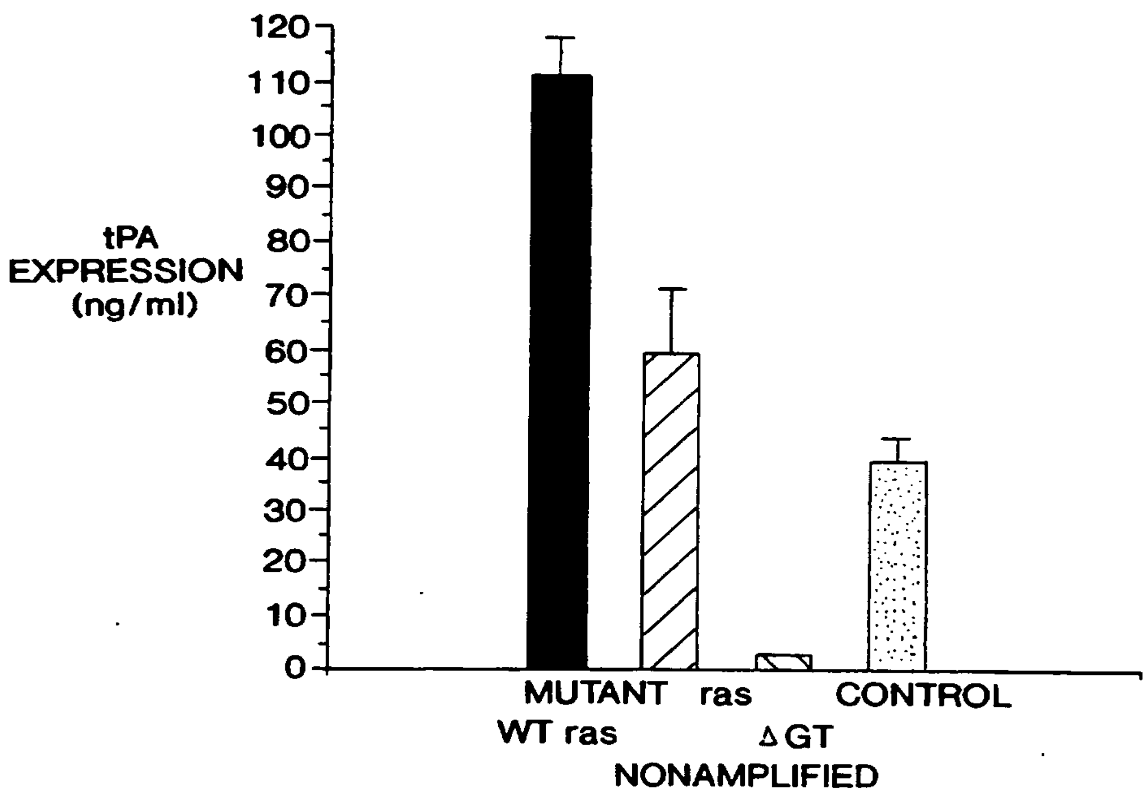

120
110
100
90
80
70
60
50
40
30
20
10
0
tPA
EXPRESSION
(ng/ml)
MUTANT
WT ras
ras
Δ GT
CONTROL
NONAMPLIFIED

FIG. 5A

FIG. 10A

```
        aluI
       sstI
       sacI
       hgiJII
       hgiAI/aspHI
       ecl136II
       bsp1286
       bsiHKAI
       bmyI                      rmaI    tru9I
       banII                     maeI    mseI                                                        thaI
     taqI                        speI   aseI/asnI/vspI                                               fnuDII/mvnJ
                                                                                                     bstUI
                                                                                                     bsh1236I
                                                                           bslI                    aciI maeIII
  1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC
    AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCAATAATTA TCATTAGTTA ATGCCCCAGT AATCAAGTAT CGGGTATATA CCTCAAGGCG CAATGTATTG

                      scrFI
                      mvaI
                      ecoRII
                      dsaV
                   aciI
                 bglI bstNI                                 maeII
                sau96I                                     hinlI/acyI
                haeIII/palI     aciI                       ahaII/bsaHI
                asuI  apyI[dcm+]               aciI        aatII         maeII           maeIII
101 TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA
    AATGCCATTT ACCGGGCGGA CCGACTGGCG GGTTGCTGGG GGCGGGTAAC TGCAGTTATT ACTGCATACA AGGGTATCAT TGCGGTTATC CCTGAAAGGT

       maeII                                                                                         maeII
      hinlI/acyI                                                                                    hinlI/acyI
      ahaII/bsaHI                                    rsaI                        rsaI               ahaII/bsaHI
      aatII                              bglI        csp6I           ndeI        csp6I              aatII
201 TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT
    AACTGCAGTT ACCCACCTCA TAAATGCCAT TTGACGGGTG AACCGTCATG TAGTTCACAT AGTATACGGT TCATGCGGGG GATAACTGCA GTTACTGCCA

               scrFI
               mvaI
               ecoRII
           aciI                                                                                         nlaIII
         bglI  dsaV                                                                                 styI
        sau96I bstNI            rsaI                                        maeII                      ncoI
        haeIII/palI             csp6I                               rsaI   snaBI                       dsaI hphI  aciI
        asuI   apyI[dcm+]    bsrI  nlaIII                           csp6I  bsaAI                       bsaJI   sfaNI
301 AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC
    TTTACCGGGC GGACCGTAAT ACGGGTCATG TACTGGAATA CCCTGAAAGG ATGAACCGTC ATGTAGATGC ATAATCAGTA GCGATAATGG TACCACTACG
```

FIG. 9R

```
                                                                  thaI
                                                                  fnuDII/mvnI
                                                                  bstUI
                                                                  bsh1236I
                                                                hinPI
                                                                hhaI/cfoI
                                                               thaI
                                                               fnuDII/mvnI
                 sapI  hinPI                                   bstUI               tru9I   aluI
                 mboII hhaI/cfoI                               bsh1236I haeIII/palI       pvuII
                 earI/ksp632I                       mnlI    bslI     eaeI  tfiI aseI/asnI/vspI
       mnlI   aciI    haeII                     aciI       aciI      cfrI  hinfI mseI   nspBII          bsrI
6301 AGTGAGCGAG GAAGCGGAAG AGCGCCCAAT ACGCAAACCG CCTCTCCCCG CGCGTTGGCC GATTCATTAA TCCAGCTGGC ACGACAGGTT TCCCGACTGG
     TCACTCGCTC CTTCGCCTTC TCGCGGGTTA TGCGTTTGGC GGAGAGGGGC GCGCAACCGG CTAAGTAATT AGGTCGACCG TGCTGTCCAA AGGGCTGACC

                                                                      scrFI
                                                                      mvaI
                                                                      ecoRII
                                                                      dsaV
                                  tru9I                          nlaIV bstNI
                     hinPI        mseI      maeIII               hgiCI apyI[dcm+]               mspI
     aciI            hhaI/cfoI   aseI/asnI/vspI mnlI             banI bsaJI                     hpaII
6401 AAAGCGGGCA GTGAGCGCAA CGCAATTAAT GTGAGTTACC TCACTCATTA GGCACCCCAG GCTTTACACT TTATGCTTCC GGCTCGTATG TTGTGTGGAA
     TTTCGCCCGT CACTCGCGTT GCGTTAATTA CACTCAATGG AGTGAGTAAT CCGTGGGGTC CGAAATGTGA AATACGAAGG CCGAGCATAC AACACACCTT

                                                                   tru9I
                                                                   mseI
                                                                  aseI/asnI/vspI
        aciI                                                     xmnI
      bsrBI                            aluI      nlaIII          asp700
6501 TTGTGAGCGG ATAACAATTT CACACAGGAA ACAGCTATGA CCATGATTAC GAATTAA
     AACACTCGCC TATTGTTAAA GTGTGTCCTT TGTCGATACT GGTACTAATG CTTAATT
```

>length: 6557

FIG. 9Q

```
                                                                                                      mspI
                                                             hinPI                                    hpaII
                                                             hhaI/cfoI                                bslI    fnu4HI
                           ddeI     scfI                    haeII                        aciI        bsaWI    aciI
5901 AGCGAACGAC CTACACCGAA CTGAGATACC TACAGCGTGA GCATTGAGAA AGCGCCACGC TTCCCGAAGG GAGAAAGGCG GACAGGTATC CGGTAAGCGG
     TCGCTTGCTG GATGTGGCTT GACTCTATGG ATGTCGCACT CGTAACTCTT TCGCGGTGCG AAGGGCTTCC CTCTTTCCGC CTGTCCATAG GCCATTCGCC

                                          scrFI
                                          mvaI          scrFI
                                          ecoRII  mvaI
                                          dsaV    ecoRII
                                          bstNI         dsaV
                        hinPI  mnlI       bsaJI         bstNI                                                 taqI
                        hhaI/cfoI   aluI  apyI[dcm+]    apyI[dcm+]                            mnlI drdI    hgaI
6001 CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG GGAAACGCCT GGTATCTTTA TAGTCCTGTC GGGTTTCGCC ACCTCTGACT TGAGCGTCGA
     GTCCCAGCCT TGTCCTCTCG CGTGCTCCCT CGAAGGTCCC CCTTTGCGGA CCATAGAAAT ATCAGGACAG CCCAAAGCGG TGGAGACTGA ACTCGCAGCT

                                                                                   haeIII/palI
                                                               haeIII/palI     scrFI
                                                             fnu4HI            mvaI
                                                             aciI              ecoRII
                                                            thaI bslI          dsaV                    nlaIII
                                                            fnuDII/mvnI        bstNI bslI                   nspI
                                nlaIV                       bstUI              apyI[dcm+]      haeIII/palI  nspHI
            sfaNI             aciI                          bsh1236I       nlaIV  haeI        haeI          aflIII
6101 TTTTTGTGAT GCTCGTCAGG GGGGCGGAGC CTATGGAAAA ACGCCAGCAA CGCGGCCTTT TTACGGTTCC TGGCCTTTTG CTGGCCTTTT GCTCACATGT
     AAAAACACTA CGAGCAGTCC CCCCGCCTCG GATACCTTTT TGCGGTCGTT GCGCCGGAAA AATGCCAAGG ACCGGAAAAC GACCGGAAAA CGAGTGTACA

                                                                                    fnu4HI              fnu4HI
                                                                                    bbvI                bbvI pleI
                           tfiI                                           bsrBI   aciI               hinPI   hinfI
                           hinfI                   aciI          aluI     aciI  fnu4HI        mcrI   hhaI/cfoI
6201 TCTTTCCTGC GTTATCCCCT GATTCTGTGG ATAACCGTAT TACCGCCTTT GAGTGAGCTG ATACCGCTCG CCGCAGCCGA ACGACCGAGC GCAGCGAGTC
     AGAAAGGACG CAATAGGGGA CTAAGACACC TATTGGCATA ATGGCGGAAA CTCACTCGAC TATGGCGAGC GGCGTCGGCT TGCTGGCTCG CGTCGCTCAG
```

FIG. 9P

```
                                                            sau3AI
                                                            mboI/ndeII[dam-]
                                                            dpnI[dam+]  sau3AI           thaI
                                                            dpnII[dam-] mboI/ndeII[dam-]
                                                           bstYI/xhoII  dpnI[dam+]       fnuDII/mvnI
                                                  sau3AI   alwI[dam-]   dpnII[dam-]      bstUI
                                                  mboI/ndeII[dam-]      alwI[dam-]       bsh1236I
                    hgaI                          dpnI[dam+] mboII[dam-]                hinPI      fnu4HI
                ddeI                              dpnII[dam-]          bstYI/xhoII      hhaI/cfoI  bbvI
5501 TTTTCGTTCC ACTGAGCGTC AGACCCCGTA GAAAAGATCA AAGGATCTTC TTGAGATCCT TTTTTTCTGC GCGTAATCTG CTGCTTGCAA ACAAAAAAAC
     AAAAGCAAGG TGACTCGCAG TCTGGGGCAT CTTTTCTAGT TTCCTAGAAG AACTCTAGGA AAAAAAGACG CGCATTAGAC GACGAACGTT TGTTTTTTTG

                                            sau3AI
                                            mboI/ndeII[dam-]
                                            dpnI[dam+]
                                            dpnII[dam-]
                                           alwI[dam-]
                acil                     mspI                                    bsrI           hinPI
      aciI   nspBII                      hpaII      aluI                     maeIII   eco57I    hhaI/cfoI
5601 CACCGCTACC AGCGGTGGTT TGTTTGCCGG ATCAAGAGCT ACCAACTCTT TTTCCGAAGG TAACTGGCTT CAGCAGAGCG CAGATACCAA ATACTGTCCT
     GTGGCGATGG TCGCCACCAA ACAAACGGCC TAGTTCTCGA TGGTTGAGAA AAAGGCTTCC ATTGACCGAA GTCGTCTCGC GTCTATGGTT TATGACAGGA

                                                                                                        fnu4HI
                                                                                                 alwNI  bbvI
     rmaI              haeIII/palI                                                             bsrI   fnu4HI
     maeI      bslI   haeI                     scfI     aciI        mnlI                    maeIII    bbvI     bsrI
5701 TCTAGTGTAG CCGTAGTTAG GCCACCACTT CAAGAACTCT GTAGCACCGC CTACATACCT CGCTCTGCTA ATCCTGTTAC CAGTGGCTGC TGCCAGTGGC
     AGATCACATC GGCATCAATC CGGTGGTGAA GTTCTTGAGA CATCGTGGCG GATGTATGGA GCGAGACGAT TAGGACAATG GTCACCGACG ACGGTCACCG

                   scrFI                                              aciI                    hgiAI/aspHI
                   nciI                                             nspBII                    bsp1286
                   mspI                               mspI         fnu4HI                     bsiHKAI
                   hpaII                              hpaII        bbvI                       bmyI
                   dsaV     pleI                     bsaWI       hinPI mcrI                   apaLI/snoI
                   cauII    hinfI                maeIII          hhaI/cfoI                    alw44I/snoI  aluI
5801 GATAAGTCGT GTCTTACCGG GTTGGACTCA AGACGATAGT TACCGGATAA GGCGCAGCGG TCGGGCTGAA CGGGGGGTTC GTGCACACAG CCCAGCTTGG
     CTATTCAGCA CAGAATGGCC CAACCTGAGT TCTGCTATCA ATGGCCTATT CCGCGTCGCC AGCCCGACTT GCCCCCCAAG CACGTGTGTC GGGTCGAACC
```

FIG. 9O

```
                                                                                               hinPI
                 mspI                                                                         mstI
                 hpaII                                                                        aviII/fspI     bsrI
                bsaWI                                                    fnu4HI          maeII hhaI/cfoI  tru9I
            nlaIV    aluI                             maeIII     sfaNI   bbvI           psp1406I          mseI
5001 ATCGTTGGGA ACCGGAGCTG AATGAAGCCA TACCAAACGA CGAGCGTGAC ACCACGATGC CAGCAGCAAT GGCAACAACG TTGCGCAAAC TATTAACTGG
     TAGCAACCCT TGGCCTCGAC TTACTTCGGT ATGGTTTGCT GCTCGCACTG TGGTGCTACG GTCGTCGTTA CCGTTGTTGC AACGCGTTTG ATAATTGACC

                            mspI
                            hpaII                                                              bglI
                           scrFI                                                              sau96I
                     aluI  nciI        tru9I      fokI                     sau96I             haeIII/palI
                   rmaI    dsaV        mseI   bsrI        aciI             avaII       hinPI  asuI   mspI
                   maeI    cauII      aseI/asnI/vspI   mnlI                asuI        hhaI/cfoI     hpaII
5101 CGAACTACTT ACTCTAGCTT CCCGGCAACA ATTAATAGAC TGGATGGAGG CGGATAAAGT TGCAGGACCA CTTCTGCGCT CGGCCCTTCC GGCTGGCTGG
     GCTTGATGAA TGAGATCGAA GGGCCGTTGT TAATTATCTG ACCTACCTCC GCCTATTTCA ACGTCCTGGT GAAGACGCGA GCCGGGAAGG CCGACCGACC

                             mspI              thaI
                             hpaII             fnuDII/mvnI              haeIII/palI
                            cfr10I             bstUI                   sau96I
                        nlaIV  hphI        bsmAI aciI        fnu4HI  nlaIV
                      gsuI/bpmI           bsaI  bsh1236I    bbvI bsrI asuI             mnlI
5201 TTTATTGCTG ATAAATCTGG AGCCGGTGAG CGTGGGTCTC GCGGTATCAT TGCAGCACTG GGGCCAGATG GTAAGCCCTC CCGTATCGTA GTTATCTACA
     AAATAACGAC TATTTAGACC TCGGCCACTC GCACCCAGAG CGCCATAGTA ACGTCGTGAC CCCGGTCTAC CATTCGGGAG GGCATAGCAT CAATAGATGT

                                                      ddeI
                                                 sau3AI        nlaIV
            pleI                                 mboI/ndeII[dam-]  mnlI
            hinfI                                dpnI[dam+]    hgiCI        tru9I
      eam1105I               fokI                dpnII[dam-]   banI         mseI       maeIII
5301 CGACGGGGAG TCAGGCAACT ATGGATGAAC GAAATAGACA GATCGCTGAG ATAGGTGCCT CACTGATTAA GCATTGGTAA CTGTCAGACC AAGTTTACTC
     GCTGCCCCTC AGTCCGTTGA TACCTACTTG CTTTATCTGT CTAGCGACTC TATCCACGGA GTGACTAATT CGTAACCATT GACAGTCTGG TTCAAATGAG

                                                         rmaI      sau3AI
                                                      sau3AI hphI  mboI/ndeII[dam-]
                                                      mboI/ndeII[dam-]
                                                      dpnI[dam+]   dpnI[dam+]
                                              tru9I   dpnII[dam-]  dpnII[dam-]
                         tru9I               ahaIII/draI maeI      alwI[dam-]         nlaIII               maeII
                         mseI           tru9I       bstYI/xhoII  bstYI/xhoII         rcaI               tru9I
                        ahaIII/draI     mseI mseI   alwI[dam-] mboII[dam-]          bspHI              mseI
5401 ATATATACTT TAGATTGATT TAAAACTTCA TTTTTAATTT AAAAGGATCT AGGTGAAGAT CCTTTTTGAT AATCTCATGA CCAAAATCCC TTAACGTGAG
     TATATATGAA ATCTAACTAA ATTTTGAAGT AAAAATTAAA TTTTCCTAGA TCCACTTCTA GGAAAAACTA TTAGAGTACT GGTTTTAGGG AATTGCACTC
```

FIG. 9N

```
                                      hgiAI/aspHI
                                      bsp1286
                         sau3AI       bsiHKAI                                    sau3AI  nspBII    sau3AI
                         mboI/ndeII[dam-]                                        mboI/ndeII[dam-]  mboI/ndeII[dam-]
                         dpnI[dam+] bmyI                                         dpnI[dam+]        dpnI[dam+]
                         dpnII[dam-]                                           bstYI/xhoII         dpnII[dam-]
                   eco57I             apaLI/snoI                            bsrI dpnII[dam-]       alwI[dam-]
     hphI         sfaNI  mboII[dam-]  alw44I/snoI  maeIII taqI   alwI[dam-]    aciI  bstYI/xhoII             mboII
4601 CTGGTGAAAG TAAAAGATGC TGAAGATCAG TTGGGTGCAC GAGTGGGTTA CATCGAACTG GATCTCAACA GCGGTAAGAT CCTTGAGAGT TTTCGCCCCG
     GACCACTTTC ATTTTCTACG ACTTCTAGTC AACCCACGTG CTCACCCAAT GTAGCTTGAC CTAGAGTTGT CGCCATTCTA GGAACTCTCA AAAGCGGGGC

                                                                            scrFI
                                                                            nciI
                                                         aciI               mspI
                                                        thaI                hpaII
                                                        fnuDII/mvnI         dsaV
       maeII             hgiAI/aspHI                    bstUI               cauII
      psp1406I           bsp1286  tru9I                 bsh1236I        hinlI/acyI
     xmnI                bsiHKAI  mseI                 hinPI           hgaI                         aciI
     asp700              bmyI    ahaIII/draI           hhaI/cfoI       ahaII/bsaHI   bcgI   mcrI  fnu4HI
4701 AAGAACGTTT TCCAATGATG AGCACTTTTA AAGTTCTGCT ATGTGGCGCG GTATTATCCC GTGATGACGC CGGGCAAGAG CAACTCGGTC GCCGCATACA
     TTCTTGCAAA AGGTTACTAC TCGTGAAAAT TTCAAGACGA TACACCGCGC CATAATAGGG CACTACTGCG GCCCGTTCTC GTTGAGCCAG CGGCGTATGT

                         rsaI
                         csp6I    bsrI                                                      fnu4HI
       ddeI             scaI hphI   maeIII      sfaNI     fokI    nlaIII                    bbvI        nlaIII
4801 CTATTCTCAG AATGACTTGG TTGAGTACTC ACCAGTCACA GAAAAGCATC TTACGGATGG CATGACAGTA AGAGAATTAT GCAGTGCTGC CATAACCATG
     GATAAGAGTC TTACTGAACC AACTCATGAG TGGTCAGTGT CTTTTCGTAG AATGCCTACC GTACTGTCAT TCTCTTAATA CGTCACGACG GTATTGGTAC

                                               sau96I
                                               avaII                                          maeIII
                                       sau3AI  asuI                                          nlaIII
              haeIII/palI              mboI/ndeII[dam-]                                     sau3AI
             eaeI                      dpnI[dam+]                                           mboI/ndeII[dam-]  sau3AI
             cfrI                      dpnII[dam-]                                          dpnI[dam+]        mboI/ndeII[dam-]
            fnu4HI                    pvuI/bspCI                                            dpnII[dam-]       dpnI[dam+]
            aciI                      mcrI   mnlI          aluI  aciI              nlaIII alwI[dam-]          dpnII[dam-]
4901 AGTGATAACA CTGCGGCCAA CTTACTTCTG ACAACGATCG GAGGACCGAA GGAGCTAACC GCTTTTTTGC ACAACATGGG GGATCATGTA ACTCGCCTTG
     TCACTATTGT GACGCCGGTT GAATGAAGAC TGTTGCTAGC CTCCTGGCTT CCTCGATTGG CGAAAAAACG TGTTGTACCC CCTAGTACAT TGAGCGGAAC
```

FIG. 9M

```
                                                                                                                  hinPI
                                                                                                                  hhaI/cfoI
                                                                                                                 thaI
                                                                                 hinPI                           fnuDII/mvnI
           aciI                                           maeIII               fnu4HI                           bstUI
          fnu4HI     tru9I                              maeII bsrI  nlaIII  hhaI/cfoI               nspBII  bsh1236I
       sfaNI         mseI          aciI                 bsaAI tth111I/aspI bbvI            aciI         aciI  hgaI
4101 ACAATCTGCT CTGATGCCGC ATAGTTAAGC CAACTCCGCT ATCGCTACGT GACTGGGTCA TGGCTGCGCC CCGACACCCG CCAACACCCG CTGACGCGCC
     TGTTAGACGA GACTACGGCG TATCAATTCG GTTGAGGCGA TAGCGATGCA CTGACCCAGT ACCGACGCGG GGCTGTGGGC GGTTGTGGGC GACTGCGCGG

                                                                                                                      thaI
                                                                                                                      fnuDII/mvnI
                                                                                                                      bstUI
                                                                 scrFI                                                bsh1236I
                  mspI                                           nciI                                                hinPI
                  hpaII                                          mspI                                                hhaI/cfoI
                 scrFI                                      bsmAI          nspI                                     thaI
                 nciI     aciI                              esp3I hpaII fnu4HI                                      fnuDII/mvnI
                 dsaV sfaNI                                bslI  dsaV aluI nspHI                                    bstUI
   drdI          cauII fokI                     aluI maeIII      cauII bbvI nlaIII    mnlI    hphI      hphI        bsh1236I
4201 CTGACGGGCT TGTCTGCTCC CGGCATCCGC TTACAGACAA GCTGTGACCG TCTCCGGGAG CTGCATGTGT CAGAGGTTTT CACCGTCATC ACCGAAACGC
     GACTGCCCGA ACAGACGAGG GCCGTAGGCG AATGTCTGTT CGACACTGGC AGAGGCCCTC GACGTACACA GTCTCCAAAA GTGGCAGTAG TGGCTTTGCG

                                 mnlI
                               haeIII/palI                                                       maeII
                mboII         sau96I                                   nlaIII                    hinlI/acyI
                bpuAI         asuI                              tru9I  rcaI                      ahaII/bsaHI
   mnlI         bbsI         eco0109I/draII                     mseI   bspHI                 ddeI aatII
4301 GCGAGGCAGT ATTCTTGAAG ACGAAAGGGC CTCGTGATAC GCCTATTTTT ATAGGTTAAT GTCATGATAA TAATGGTTTC TTAGACGTCA GGTGGCACTT
     CGCTCCGTCA TAAGAACTTC TGCTTTCCCG GAGCACTATG CGGATAAAAA TATCCAATTA CAGTACTATT ATTACCAAAG AATCTGCAGT CCACCGTGAA

                 nlaIV
                aciI
               thaI
               fnuDII/mvnI
               bstUI                                                       rcaI
               bsh1236I                                                    bspHI
              hinPI                                                     bsrBI    bsmAI
              hhaI/cfoI                                                 aciI nlaIII                              sspI
4401 TTCGGGGAAA TGTGCGCGGA ACCCCTATTT GTTTATTTTT CTAAATACAT TCAAATATGT ATCCGCTCAT GAGACAATAA CCCTGATAAA TGCTTCAATA
     AAGCCCCTTT ACACGCGCCT TGGGGATAAA CAAATAAAAA GATTTATGTA AGTTTATACA TAGGCGAGTA CTCTGTTATT GGGACTATTT ACGAAGTTAT

             mboII                                                     fnu4HI
             earI/ksp632I                                              aciI                     hphI
4501 ATATTGAAAA AGGAAGAGTA TGAGTATTCA ACATTTCCGT GTCGCCCTTA TTCCCTTTTT TGCGGCATTT TGCCTTCCTG TTTTTGCTCA CCCAGAAACG
     TATAACTTTT TCCTTCTCAT ACTCATAAGT TGTAAAGGCA CAGCGGGAAT AAGGGAAAAA ACGCCGTAAA ACGGAAGGAC AAAAACGAGT GGGTCTTTGC
```

FIG. 9L

```
                                                                   aciI
                                    hinPI                       fnu4HI                  fnu4HI
                                    hhaI/cfoI                   aciI                  hinPI
                                   thaI                        thaI                   hhaI/cfoI
                                   fnuDII/mvnI                 fnuDII/mvnI           thaI
                                   bstUI          hinPI        bstUI                 fnuDII/mvnI
                                   bsh1236I       hhaI/cfoI hinPI                    bstUI                   hinPI
                                rsaI      scfI  fnu4HI   tru9I bsh1236I              bsh1236I     aciI        hhaI/cfoI
       aciI  maeII              csp6I   bslI   aciI      mseI hhaI/cfoI         maeIII  bbvI  maeIII       haeII
3601 CACCGCATAC GTCAAAGCAA CCATAGTACG CGCCCTGTAG CGGCGCATTA AGCGCGGCGG GTGTGGTGGT TACGCGCAGC GTGACCGCTA CACTTGCCAG
     GTGGCGTATG CAGTTTCGTT GGTATCATGC GCGGGACATC GCCGCGTAAT TCGCGCCGCC CACACCACCA ATGCGCGTCG CACTGGCGAT GTGAACGGTC

            hinPI                                                                                nlaIV
            hhaI/cfoI                                          mspI                             hgiJII
           haeII                                               hpaII                            bsp1286
         rmaI    bsrBI                                        naeI                              bmyI
         maeI    aciI             mboII                 maeII  cfr10I            aluI           banII         nlaIV
3701 CGCCCTAGCG CCCGCTCCTT TCGCTTTCTT CCCTTCCTTT CTCGCCACGT TCGCCGGCTT TCCCCGTCAA GCTCTAAATC GGGGGCTCCC TTTAGGGTTC
     GCGGGATCGC GGGCGAGGAA AGCGAAAGAA GGGAAGGAAA GAGCGGTGCA AGCGGCCGAA AGGGGCAGTT CGAGATTTAG CCCCCGAGGG AAATCCCAAG

                      nlaIV                                            maeII    haeIII/palI
                      hgiCI  taqI                                     draIII   sau96I                                maeII
                      banI mnlI                  hphI                 bsaAI    asuI                                 drdI
3801 CGATTTAGTG CTTTACGGCA CCTCGACCCC AAAAAACTTG ATTTGGGTGA TGGTTCACGT AGTGGGCCAT CGCCCTGATA GACGGTTTTT CGCCCTTTGA
     GCTAAATCAC GAAATGCCGT GGAGCTGGGG TTTTTTGAAC TAAACCCACT ACCAAGTGCA TCACCCGGTA GCGGGACTAT CTGCCAAAAA GCGGGAAACT

          pleI          tru9I     pleI                                   bslI
          hinfI  maeII  mseI      hinfI           bsrI                  bslI   avaI
3901 CGTTGGAGTC CACGTTCTTT AATAGTGGAC TCTTGTTCCA AACTGGAACA ACACTCAACC CTATCTCGGG CTATTCTTTT GATTTATAAG GGATTTTGCC
     GCAACCTCAG GTGCAAGAAA TTATCACCTG AGAACAAGGT TTGACCTTGT TGTGAGTTGG GATAGAGCCC GATAAGAAAA CTAAATATTC CCTAAAACGG

                                                          thaI                                          hgiAI/aspHI
                                                          fnuDII/mvnI                                   bsp1286
                                                          bstUI                    maeII                bsiHKAI
                      tru9I                         tru9I                    tru9I                      bmyI    ddeI
                      mseI              tru9I       mseI    apoI  tru9I      mseI                       apaLI/snoI  rsaI
           haeIII/palI          aluI    mseI    apoI     bsh1236I mseI    sspI  psp1406I                alw44I/snoI csp6I
4001 GATTTCGGCC TATTGGTTAA AAAATGAGCT GATTTAACAA AAATTTAACG CGAATTTTAA CAAAATATTA ACGTTTACAA TTTTATGGTG CACTCTCAGT
     CTAAAGCCGG ATAACCAATT TTTTACTCGA CTAAATTGTT TTTAAATTGC GCTTAAAATT GTTTTATAAT TGCAAATGTT AAAATACCAC GTGAGAGTCA
```

FIG. 9K

```
                                                                      fnu4HI
                         nlaIII                                       sfiI mnlI
                        styI                                          haeIII/palI
                        ncoI                                     bsaJI bglI        ddeI
                   bslI dsaI                                   haeIII/palI bsaJI  mnlI  aluI          mnlI
     bsrI  aciI    aciI bsaJI                                 mnlI  mnlI aciI   haeIII/palI          mnlI
3201 CCAGTTCCGC CCATTCTCCG CCCCATGGCT GACTAATTTT TTTTATTTAT GCAGAGGCCG AGGCCGCCTC GGCCTCTGAG CTATTCCAGA AGTAGTGAGG
     GGTCAAGGCG GGTAAGAGGC GGGGTACCGA CTGATTAAAA AAAATAAATA CGTCTCCGGC TCCGGCGGAG CCGGAGACTC GATAAGGTCT TCATCACTCC

                                                         fnu4HI          hinPI
                 rmaI                                   mcrI             hhaI/cfoI
                styI                                    eagI/xmaIII/eclXI thaI
                bsaJI                                   eaeI             fnuDII/mvnI
                blnI                                 bsrBI        tru9I bstUI            bspMI
                avrII                              xhoI notI    tru9I   bsh1236I       scfI
              haeIII/palI                          paeR7I haeIII/palI  hinPI    tru9I  pstI
              stuI                                 avaI fnu4HI  pacI   hhaI/cfoI mseI  bsgI
              haeI                            maeIII taqI cfrI  mseI   bssHII  ahaIII/draI   maeIII
             mnlI maeI                   aluI     mnlI  aciI aciI  mseI ascI   swaI   sse8387I   aluI   bsrI
3301 AGGCTTTTTT GGAGGCCTAG GCTTTTGCAA AAAGCTGTTA CCTCGAGCGG CCGCTTAATT AAGGCGCGCC ATTTAAATCC TGCAGGTAAC AGCTTGGCAC
     TCCGAAAAAA CCTCCGGATC CGAAAACGTT TTTCGACAAT GGAGCTCGCC GGCGAATTAA TTCCGCGCGG TAAATTTAGG ACGTCCATTG TCGAACCGTG

                                            scrFI
                                            mvaI
                                            ecoRII
                                            dsaV
      haeIII/palI                           bstNI                                               aluI
     eaeI                     bsrI          apyI[dcm+]       tru9I      fnu4HI                 pvuII          mboII
     cfrI            maeII maeIII         bsaJI   maeIII     mseI       bbvI  fokI             nspBII         earI/ksp632I
3401 TGGCCGTCGT TTTACAACGT CGTGACTGGG AAAACCCTGG CGTTACCCAA CTTAATCGCC TTGCAGCACA TCCCCCCTTC GCCAGCTGGC GTAATAGCGA
     ACCGGCAGCA AAATGTTGCA GCACTGACCC TTTTGGGACC GCAATGGGTT GAATTAGCGG AACGTCGTGT AGGGGGGAAG CGGTCGACCG CATTATCGCT

                                                              hinPI
                                                              hhaI/cfoI
                                                             nlaIV
                 sau3AI                                      narI
                 mboI/ndeII[dam-]                            kasI
                 dpnI[dam+]                                  hinlI/acyI
        sau96I   dpnII[dam-]                                 hgiCI
        haeIII/palI                                          haeII     aciI
        asuI    pvuI/bspCI                                   banI   sfaNI
      mnlI aciI mcrI                     bglI                ahaII/bsaHI                  sfaNI  aciI
3501 AGAGGCCCGC ACCGATCGCC CTTCCCAACA GTTGCGTAGC CTGAATGGCG AATGGCGCCT GATGCGGTAT TTTCTCCTTA CGCATCTGTG CGGTATTTCA
     TCTCCGGGCG TGGCTAGCGG GAAGGGTTGT CAACGCATCG GACTTACCGC TTACCGCGGA CTACGCCATA AAAGAGGAAT GCGTAGACAC GCCATAAAGT
```

FIG. 9J

```
                                                                                                                              taqI(dam-)
                                                                                                                               claI/bsp106(dam-)
                                                                                                                              sau3AI
                                                                                                                              mboI/ndeII(dam-)
                                                                                                                              dpnI(dam+)
                                                                  rmaI                                                        dpnII(dam-)
          sfaNI    apoI                                    bsmI   maeI                                                 nlaIII  alwI(dam-)
2801 AAAGCAATAG CATCACAAAT TTCACAAATA AAGCATTTTT TTCACTGCAT TCTAGTTGTG GTTTGTCCAA ACTCATCAAT GTATCTTATC ATGTCTGGAT
     TTTCGTTATC GTAGTGTTTA AAGTGTTTAT TTCGTAAAAA AAGTGACGTA AGATCAACAC CAAACAGGTT TGAGTAGTTA CATAGAATAG TACAGACCTA

              tru9I                                                                rsaI
          xmnI                                                                     csp6I
     sau3AI  aseI/asnI/vspI     styI                                               nlaIV
     mboI/ndeII(dam-)           ncoI                                               kpnI
     dpnI(dam+)            fnu4HI   haeI                                           hgiCI
     dpnII(dam-)           bbvI  dsaI haeIII/palI                                  banI                     aluI
     pvuI/bspCI mseI     hinPI    bsaJI                                            asp718     mnlI          pvuII
     mcrI   asp700       hhaI/cfoI nlaIII          mnlI       mnlI                 acc65I  ddeI aciI        nspBII
2901 CGATCGGGAA TTAATTCGGC GCAGCACCAT GGCCTGAAAT AACCTCTGAA AGAGGAACTT GGTTAGGTAC CTTCTGAGGC GGAAAGAACC AGCTGTGGAA
     GCTAGCCCTT AATTAAGCCG CGTCGTGGTA CCGGACTTTA TTGGAGACTT TCTCCTTGAA CCAATCCATG GAAGACTCCG CCTTTCTTGG TCGACACCTT

                                nlaIV
                             scrFI                                                         scrFI
                             mvaI                                  ppu10I                       mvaI
                             ecoRII                             nsiI/avaIII                ecoRII
                             dsaV                              nlaIII                      dsaV
                             bstNI                                sphI                          bstNI
                             apyI(dcm+)                           nspI sfaNI                    apyI(dcm+)
                            bsaJI                                 nspHI                        sexAI              bsaJI
3001 TGTGTGTCAG TTAGGGTGTG GAAAGTCCCC AGGCTCCCCA GCAGGCAGAA GTATGCAAAG CATGCATCTC AATTAGTCAG CAACCAGGTG TGGAAAGTCC
     ACACACAGTC AATCCCACAC CTTTCAGGGG TCCGAGGGGT CGTCCGTCTT CATACGTTTC GTACGTAGAG TTAATCAGTC GTTGGTCCAC ACCTTTCAGG

       nlaIV                         sfaNI
     scrFI                        ppu10I
     mvaI                              nsiI/avaIII
     ecoRII                      nlaIII
     dsaV                       sphI
     bstNI                            nspI                                                        aciI
     apyI(dcm+)                       nspHI                             aciI          aciI   fokI              aciI
3101 CCAGGCTCCC CAGCAGGCAG AAGTATGCAA AGCATGCATC TCAATTAGTC AGCAACCATA GTCCCGCCCC TAACTCCGCC CATCCCGCCC CTAACTCCGC
     GGTCCGAGGG GTCGTCCGTC TTCATACGTT TCGTACGTAG AGTTAATCAG TCGTTGGTAT CAGGGCGGGG ATTGAGGCGG GTAGGGCGGG GATTGAGGCG
```

FIG. 9I

```
                                           scrFI
                                           nciI
                                           mspI
                                           hpaII
                                           dsaV
                                           cauII
                                          xmaI/pspAI
                                          smaI
                                          scrFI                   scrFI
                                          nciI                    mvaI                scrFI
                                          dsaV                    ecoRII        mvaI
                                          cauII                   dsaV          ecoRII
                       rsaI            fokI    mboII              bstNI               dsaV
                       csp6I       bslI  bsaJI                    apyI[dcm+]          bstNI
      avaI            bsp1407I     bslI   avaI earI/ksp632I      sexAI          bspMI apyI[dcm+]
2401 CCCCGAGAAC CACAGGTGTA CACCCTGCCC CCATCCCGGG AAGAGATGAC CAAGAACCAG GTCAGCCTGA CCTGCCTGGT CAAAGGCTTC TATCCCAGCG
     GGGGCTCTTG GTGTCCACAT GTGGGACGGG GGTAGGGCCC TTCTCTACTG GTTCTTGGTC CAGTCGGACT GGACGGACCA GTTTCCGAAG ATAGGGTCGC

           dsaI                     mspI
           bslI                fnu4HI                                      pleI                  mnlI
           bsaJI               bbvI hpaII                    mnlI          hinfI    nlaIV   mboII   scfI    aluI
2501 ACATCGCCGT GGAGTGGGAG AGCAATGGGC AGCCGGAGAA CAACTACAAG ACCACGCCTC CCGTGCTGGA CTCCGACGGC TCCTTCTTCC TCTACAGCAA
     TGTAGCGGCA CCTCACCCTC TCGTTACCCG TCGGCCTCTT GTTGATGTTC TGGTGCGGAG GGCACGACCT GAGGCTGCCG AGGAAGAAGG AGATGTCGTT

                                             bpuAI
                                             bbsI                   nlaIII
          dsaI                             maeII                ppu10I                           sapI
          bsaJI                fnu4HI    xmnI mboII             nsiI/avaIII                      mboII  mnlI
       hphI            bspMI   bbvI      asp700      nlaIII    sfaNI   mnlI                      earI/ksp632I bslI
2601 GCTCACCGTG GACAAGAGCA GGTGGCAGCA GGGGAACGTC TTCTCATGCT CCGTGATGCA TGAGGCTCTG CACAACCACT ACACGCAGAA GAGCCTCTCC
     CGAGTGGCAC CTGTTCTCGT CCACCGTCGT CCCCTTGCAG AAGAGTACGA GGCACTACGT ACTCCGAGAC GTGTTGGTGA TGTGCGTCTT CTCGGAGAGG

                                                                   styI
           scrFI                           taqI                 aciI
           nciI                           salI                 fnu4HI  sau96I
           mspI                    rmaI  hincII/hindII      sfiI ncoI haeIII/palI
           hpaII                   maeI  accI   scfI         haeIII/palI
           dsaV             sau96I  pleI    pstI        eaeI dsaI asuI                  aluI
           cauII            haeIII/palI     bsgI  aluI cfrI  bsaJI                  fnu4HI
       bsmAI                asuI    hinfI bspMI   hindIII bglI nlaIII               bbvI       maeIII
2701 CTGTCTCCGG GTAAATGAGT GCGACGGCCC TAGAGTCGAC CTGCAGAAGC TTGGCCGCCA TGGCCCAACT TGTTTATTGC AGCTTATAAT GGTTACAAAT
     GACAGAGGCC CATTTACTCA CGCTGCCGGG ATCTCAGCTG GACGTCTTCG AACCGGCGGT ACCGGGTTGA ACAAATAACG TCGAATATTA CCAATGTTTA
```

FIG. 9H

```
                                           sau96I
                                           nlaIV
                                           avaII
                                         mspI
                                         scrFI
                                         nciI
                                   sau3AI hpaII
                                   mboI/ndeII[dam-]
                                   dpnI[dam+]                 nlaIII
                                nlaIII   dsaV        mnlI   nspI                              drdI  mnlI
                               rcaI      cauII      ddeI maeIII                           mboII   ddeI
                   styI        bspHI[dam-]  asuI   eco81I    nspHI                        bpuAI  eco81I
                   bsaJI     mnlI  dpnII[dam-]    bsu36I/mstII/sauI        maeII          bbsI   bsu36I/mstII/sauI
2101 TTCCCCCCAA AACCCAAGGA CACCCTCATG ATCTCCCGGA CCCCTGAGGT CACATGCGTG GTGGTGGACG TGAGCCACGA AGACCCTGAG GTCAAGTTCA
     AAGGGGGGTT TTGGGTTCCT GTGGGAGTAC TAGAGGGCCT GGGGACTCCA GTGTACGCAC CACCACCTGC ACTCGGTGCT TCTGGGACTC CAGTTCAAGT

                                                       aciI
                                                      thaI
                                                      fnuDII/mvnI
                                                      bstUI
                                                      bsh1236I
                                                     sacII/sstII
                                                     nspBII
                                                     kspI
         maeII                                       dsaI                       rsaI
       rsaI                                          bsaJI                      csp6I
       csp6I                                         aciI           rsaI      maeII                 mnlI      ecoNI
     bsrI bsaAI           mnlI                      fnu4HI  mnlI    csp6I    bsaAI               hgaI   hphI   bslI
2201 ACTGGTACGT GGACGGCGTG GAGGTGCATA ATGCCAAGAC AAAGCCGCGG GAGGAGCAGT ACAACAGCAC GTACCGTGTG GTCAGCGTCC TCACCGTCCT
     TGACCATGCA CCTGCCGCAC CTCCACGTAT TACGGTTCTG TTTCGGCGCC CTCCTCGTCA TGTTGTCGTG CATGGCACAC CAGTCGCAGG AGTGGCAGGA

       scrFI
       mvaI bsrI
       ecoRII
       dsaV
       bstNI                rsaI       bsmAI                                                                fnu4HI
       apyI[dcm+]           csp6I     bsaI            mnlI         taqI                                     bbvI
2301 GCACCAGGAC TGGCTGAATG GCAAGGAGTA CAAGTGCAAG GTCTCCAACA AAGCCCTCCC AGCCCCCATC GAGAAAACCA TCTCCAAAGC CAAAGGGCAG
     CGTGGTCCTG ACCGACTTAC CGTTCCTCAT GTTCACGTTC CAGAGGTTGT TTCGGGAGGG TCGGGGGTAG CTCTTTTGGT AGAGGTTTCG GTTTCCCGTC
```

FIG. 9G

```
          scrFI                                            hinPI
          mvaI                                                  nlaIV
          ecoRII                                          narI
          ecoNI                                                 kasI                 hgiAI/aspHI
          dsaV                    hphI                    hinlI/acyI           bsp1286      mspI
          bstNI                       mspI                      hgiCI                bsiHKAI      hpaII
          bslI                        hpaII                     haeII                bmyI        scrFI
          apyI[dcm+]                cfr10I                      banI           fnu4HI            nciI
      fnu4HI                        bsaWI  tth111I/aspI         ahaII/bsaHI    aciI  apaLI/snoI  dsaV
      bbvI                    bslI  ageI maeIII             ddeI hhaI/cfoI   nspBII  alw44I/snoI cauII      scfI
1801 GGCTGCCTGG TCAAGGACTA CTTCCCCGAA CCGGTGACGG TGTCGTGGAA CTCAGGCGCC CTGACCAGCG GCGTGCACAC CTTCCCGGCT GTCCTACAGT
     CCGACGGACC AGTTCCTGAT GAAGGGGCTT GGCCACTGCC ACAGCACCTT GAGTCCGCGG GACTGGTCGC CGCACGTGTG GAAGGGCCGA CAGGATGTCA

                                                               nlaIV
      ddeI pleI                                       fnu4HI   hgiCI
     mnlI  hinfI                               mnlI   bbvI     banI                         tfiI
     eco81I           mnlI  fnu4HI  maeIII bsp1286 rmaI       bsp1286                       hinfI                styI
     bsu36I/mstII/sauI ddeI bbvI   hphI    bmyI    maeI aluI  bmyI                      maeII                bsaJI
1901 CCTCAGGACT CTACTCCCTC AGCAGCGTGG TGACTGTGCC CTCTAGCAGC TTGGGCACCC AGACCTACAT CTGCAACGTG AATCACAAGC CCAGCAACAC
     GGAGTCCTGA GATGAGGGAG TCGTCGCACC ACTGACACGG GAGATCGTCG AACCCGTGGG TCTGGATGTA GACGTTGCAC TTAGTGTTCG GGTCGTTGTG

                                                                                                 eam1105I
                                                                                                sau96I
                                                                                        scrFI
                                                                                        mvaI    avaII
                                                                                        ecoRII
                                                                                        dsaV
                         hgiJII
                         bsp1286                        nlaIII                          bstNI   asuI       mboII mboII
                         bmyI                          nspI         bsp1286             bsaJI  nlaIV      bpuAI earI/ksp632I
                         banII          maeIII         nspHI        bmyI  alwNI         apyI[dcm+]        bbsI   mnlI
2001 CAAGGTGGAC AAGAAAGTTG AGCCCAAATC TTGTGACAAA ACTCACACAT GCCCACCGTG CCCAGCACCT GAACTCCTGG GGGGACCGTC AGTCTTCCTC
     GTTCCACCTG TTCTTTCAAC TCGGGTTTAG AACACTGTTT TGAGTGTGTA CGGGTGGCAC GGGTCGTGGA CTTGAGGACC CCCCTGGCAG TCAGAAGGAG
```

FIG. 9F

```
                                                                                       scrFI
                                                                                       mvaI
                                                                                       ecoRII
                                                                                       dsaV
                                                                                       bstNI
                                                                                       apyI[dcm+]
                                                                                    hinPI
                                                                                    hhaI/cfoI
                                                                mnlI               nlaIV
                                                             xhoI                  narI
                                                             paeR7I                kasI
                                                             avaI                  hinlI/acyI
       scfI                                              hgiAI/aspHI               hgiCI
       pstI                                              bsp1286                   haeII
       bsgI                      mnlI                    bsiHKAI   fnu4HI          banI
     bspMI                     ddeI drdI                 bmyI taqI bbvI            ahaII/bsaHI
1601 ACCTGCAGAT GAACAGCCTG CGTGCTGAGG ACACTGCCGT CTATTATTGT GCTCGAGGCA GCCACTATTT CGGCGCCTGG CACTTCGCCG TGTGGGGTCA
     TGGACGTCTA CTTGTCGGAC GCACGACTCC TGTGACGGCA GATAATAACA CGAGCTCCGT CGGTGATAAA GCCGCGGACC GTGAAGCGGC ACACCCCAGT

                                             sau96I
                                             haeIII/palI
                                            sau96I                                                            scrFI
                                            nlaIV                                                             mvaI
                                            hgiJII                 nlaIV                                      ecoRII
                                            bsp1286                hgiCI                                      dsaV
           scrFI                      bsp120I               banI                                        bstNI
           mvaI                             bmyI               scrFI                                    bsaJI
           ecoRII                     banII               mvaI                                       sau96I
           dsaV                       asuI                ecoRII                               haeIII/palI
           bstNI hphI                       apaI        mboII   dsaV          hgiAI/aspHI            asuI
           apyI[dcm+] bsmAI    haeIII/palI eco0109I/draII   bstNI               bsp1286              fnu4HI
          bsaJI maeIII     mnlI         styI asuI     bpuAI    apyI[dcm+]  mnlI    bsiHKAI      bsp1286 aciI apyI[dcm+]
      nlaIV   bstEII esp3I bsaJI mnlI   bsaJI         bbsI    bsaJI     mnlI       bmyI mnlI    bmyI nspBII bsaJI
1701 AGGAACCCTG GTCACCGTCT CCTCGGCCTC CACCAAGGGC CCATCGGTCT TCCCCCTGGC ACCCTCCTCC AAGAGCACCT CTGGGGGCAC AGCGGCCCTG
     TCCTTGGGAC CAGTGGCAGA GGAGCCGGAG GTGGTTCCCG GGTAGCCAGA AGGGGGACCG TGGGAGGAGG TTCTCGTGGA GACCCCCGTG TCGCCGGGAC
```

FIG. 9E

```
                                                                                                      scrFI
                                                                                                      nciI
                                                                                                      mspI
                                                                                                      hpaII
                                                                                                      dsaV
                                                                                                      cauII
                                                                                                      bslI
                                                                                                     xmaI/pspAI
                                                                                                     smaI
                                                                                                     scrFI
                                                                                                     nciI
                                                                                                     dsaV
                                                                                                     cauII        scrFI
                                                                                                  sau96I          mvaI
                                                                                         sau3AI   nlaIV           ecoRII
         hgiJII                                                                          mboI/ndeII[dam-]         dsaV
         bsp1286                                                      mspI           dpnI[dam+]  bslI        bstNI
         bmyI                                                   hpaII                    dpnII[dam-]  bsaJI       bslI
    scrFI                                                            mroI          nlaIV     haeIII/palI     apyI[dcm+]
    mvaI banII                                                 bspMII             bstYI/xhoII  avaI      sau96I
    ecoRII                                                     bspEI                    bamHI   eco0109I/draII haeIII/palI
    dsaV                                                            bsaWI               alwI[dam-] asuI        asuI
    bstNI                                                         mnlI             bsrI alwI[dam-]  bsaJI     eco0109I/draII
    bsaJI
    apyI[dcm+]                                 alwNI           hphI  accIII    aluI
1401 GCCAGGGGGC TCACTCCGTT TGTCCTGTGC AGTTTCTGGC TACTCCATCA CCTCCGGATA TAGCTGGAAC TGGATCCGTC AGGCCCCGGG TAAGGGCCTG
     CGGTCCCCCG AGTGAGGCAA ACAGGACACG TCAAAGACCG ATGAGGTAGT GGAGGCCTAT ATCGACCTTG ACCTAGGCAG TCCGGGGCCC ATTCCCGGAC

                                         sau3AI
                                         mboI/ndeII[dam-]
                                         dpnI[dam+]                                              thaI
                              maeII      dpnII[dam-]                                             fnuDII/mvnI
                         taqI snaBI     alwI[dam-]             hgaI      haeIII/palI             bstUI  tfiI
                       claI/bsp106     mspI                  rmaI       sau96I                   bsh1236I
                   sfaNI     bsaAI     hpaII taqI[dam-]      maeI       asuI                   nruI    hinfI
1501 GAATGGGTTG CATCGATTAC GTATGCCGGA TCGACTAACT ATAACCCTAG CGTCAAGGGC CGTATCACTA TAAGTCGCGA CGATTCCAAA AACACATTCT
     CTTACCCAAC GTAGCTAATG CATACGGCCT AGCTGATTGA TATTGGGATC GCAGTTCCCG GCATAGTGAT ATTCAGCGCT GCTAAGGTTT TTGTGTAAGA
```

FIG. 9D

```
                                                                     sau96I
                                                                     avaII
                                                                     asuI
                                                                  scrFI
                                                                  mvaI
                                                                  ecoRII
                                                                  dsaV
                                                                  bstNI                              ecoRI
            maeIII                                                apyI[dcm+]      mnlI        taqI   apoI
           hphI    scfI        fokI                         bslI  bsaJI           bsaJI      claI/bsp106
1201 ATACGATTTA GGTGACACTA TAGATAACAT CCACTTTGCC TTTCTCTCCA CAGGTGTCCA CTCCCAGGTC CAACTGCACC TCGGTTCTAT CGATTGAATT
     TATGCTAAAT CCACTGTGAT ATCTATTGTA GGTGAAACGG AAAGAGAGGT GTCCACAGGT GAGGGTCCAG GTTGACGTGG AGCCAAGATA GCTAACTTAA

                                                                                                scrFI
       nlaIII                                                                                   mvaI
      styI                                                                                      ecoRII
      pflMI                                                                                     dsaV
      ncoI                                                                                      bstNI fnu4HI
      dsaI                                                   rsaI            aluI               apyI[dcm+]
      bslI fokI                        rmaI             gsuI/bpmI           pvuII    pleI      haeI     bbvI
      bsaJI       nlaIII  fokI         maeI            bsrI  csp6I          nspBII   hinfI  aciI haeIII/palI
1301 CCACCATGGG ATGGTCATGT ATCATCCTTT TTCTAGTAGC AACTGCAACT GGAGTACATT CAGAAGTTCA GCTGGTGGAG TCTGGCGGTG GCCTGGTGCA
     GGTGGTACCC TACCAGTACA TAGTAGGAAA AAGATCATCG TTGACGTTGA CCTCATGTAA GTCTTCAAGT CGACCACCTC AGACCGCCAC CGGACCACGT
```

FIG. 9C

```
                                                                                               haeIII/palI
                                                                                              haeI
                                                                       scrFI        scrFI
                                                                       mvaI                mvaI
                                                                       ecoRII  ecoRII
         mspI                                                          dsaV   tfiI   dsaV
         hpaII                                                         bstNI    nlaIII     bstNI     ddeI pleI
        bsaWI                accI nlaIII             mnlI              apyI[dcm+]   hinfI  apyI[dcm+]    hinfI
 801 ACAACCGGAA TTGGCAAGTA AAGTAGACAT GGTTTGGATA GTCGGAGGCA GTTCTGTTTA CCAGGAAGCC ATGAATCAAC CAGGCCACCT TAGACTCTTT
     TGTTGGCCTT AACCGTTCAT TTCATCTGTA CCAAACCTAT CAGCCTCCGT CAAGACAAAT GGTCCTTCGG TACTTAGTTG GTCCGGTGGA ATCTGAGAAA

                                                                                                     hgaI
                                                                                                    hinlI/acyI
                                                                                                    ahaII/bsaHI
                                                                                                 scrFI
                 nlaIII                                                                          mvaI     mnlI
             sau3AI                                                                              ecoRII
             mboI/ndeII[dam-]                                                                    dsaV
             dpnI[dam+]                  maeII                                                   bstNI    ecoNI
             dpnII[dam-]               aflIII                                                    apyI[dcm+]     mnlI
     maeIII alwI[dam-]  apoI       maeIII                                      mnlI            bsaJI     bslI ddeI
 901 GTGACAAGGA TCATGCAGGA ATTTGAAAGT GACACGTTTT TCCCAGAAAT TGATTTGGGG AAATATAAAC CTCTCCCAGA ATACCCAGGC GTCCTCTCTG
     CACTGTTCCT AGTACGTCCT TAAACTTTCA CTGTGCAAAA AGGGTCTTTA ACTAAACCCC TTTATATTTG GAGAGGGTCT TATGGGTCCG CAGGAGAGAC

         scrFI
         mvaI
         ecoRII
         dsaV
         bstNI
         apyI[dcm+]
      sau96I
      avaII                                                           sfaNI
      asuI   mnlI       sfaNI                 accI     mboII            mboII                      mnlI     aluI
1001 AGGTCCAGGA GGAAAAAGGC ATCAAGTATA AGTTTGAAGT CTACGAGAAG AAAGACTAAC AGGAAGATGC TTTCAAGTTC TCTGCTCCCC TCCTAAAGCT
     TCCAGGTCCT CCTTTTTCCG TAGTTCATAT TCAAACTTCA GATGCTCTTC TTTCTGATTG TCCTTCTACG AAAGTTCAAG AGACGAGGGG AGGATTTCGA
                                                     styI
                                                     bsaJI
                                               sau3AI
                       nlaIII                  mboI/ndeII[dam-]
                      styI                     dpnI[dam+]
                      ncoI                     dpnII[dam-]                aluI     tru9I
     ppu10I           dsaI                     alwI[dam-]               fnu4HI     mseI
     nsiI/avaIII      bsaJI                   bstYI/xhoII               bbvI      aseI/asnI/vspI
1101 ATGCATTTTT ATAAGACCAT GGGACTTTTG CTGGCTTTAG ATCCCCTTGG CTTCGTTAGA ACGCAGCTAC AATTAATACA TAACCTTATG TATCATACAC
     TACGTAAAAA TATTCTGGTA CCCTGAAAAC GACCGAAATC TAGGGGAACC GAAGCAATCT TGCGTCGATG TTAATTATGT ATTGGAATAC ATAGTATGTG
```

FIG. 9B

```
                            tfiI
    scrFI                   hinfI
    nciI                 aciI
    mspI                thaI                                                              fnu4HI
    hpaII               fnuDII/mvnI                      aciI                             bbvI
    dsaV                bstUI                   maeII rsaI                              nspBII
    cauII               bsh1236I             maeIII   csp6I  scfI          mnlI         aciI      nlaIII
401 CCGGGAACGG TGCATTGGAA CGCGGATTCC CCGTGCCAAG AGTGACGTAA GTACCGCCTA TAGAGCGATA AGAGGATTTT ATCCCCGCTG CCATCATGGT
    GGCCCTTGCC ACGTAACCTT GCGCCTAAGG GGCACGGTTC TCACTGCATT CATGGCGGAT ATCTCGCTAT TCTCCTAAAA TAGGGGCGAC GGTAGTACCA

                                                                       haeIII/palI
                                                                      haeI
                                                                     scrFI
                                                                     mvaI    bsrBI
                                                                     ecoRII
                                                                     dsaV
                                                                     bstNI   aciI                   rsaI
                                  pflMI                     bsmAI    apyI[dcm+]     xmnI            csp6I
    taqI          sfaNI           bslI                      bsaI    bsaJI mnlI  ddeI asp700         scaI
501 TCGACCATTG AACTGCATCG TCGCCGTGTC CCAAAATATG GGGATTGGCA AGAACGGAGA CCTACCCTGG CCTCCGCTCA GGAACGAGTT CAAGTACTTC
    AGCTGGTAAC TTGACGTAGC AGCGGCACAG GGTTTTATAC CCCTAACCGT TCTTGCCTCT GGATGGGACC GGAGGCGAGT CCTTGCTCAA GTTCATGAAG

                                                                    scrFI
                                                                    mvaI
                                                                    ecoRII
                      eco57I                                        dsaV
                     mboII               tfiI                       bstNI                 tfiI      tru9I
                    earI/ksp632I         hinfI                      apyI[dcm+]            hinfI     mseI
                   mnlI                alwNI    hphI               sexAI          ddeI mboII taqI  ahaIII/draI
601 CAAAGAATGA CCACAACCTC TTCAGTGGAA GGTAAACAGA ATCTGGTGAT TATGGGTAGG AAAACCTGGT TCTCCATTCC TGAGAAGAAT CGACCTTTAA
    GTTTCTTACT GGTGTTGGAG AAGTCACCTT CCATTTGTCT TAGACCACTA ATACCCATCC TTTTGGACCA AGAGGTAAGG ACTCTTCTTA GCTGGAAATT

                                                       sstI
                                                       sacI
                                                       hgiJII
                                                       hgiAI/aspHI
                                                       ecl136II
                                                       bsp1286
                                                       bsiHKAI
            tru9I                                      bmyI                                  tru9I
            mseI        ddeI                           banII                                aflII/bfrI
           aseI/asnI/vspI                    bslI  mnlI aluI         bstXI     fokI sfaNI mseI
701 AGGACAGAAT TAATATAGTT CTCAGTAGAG AACTCAAAGA ACCACCACGA GGAGCTCATT TTCTTGCCAA AAGTTTGGAT GATGCCTTAA GACTTATTGA
    TCCTGTCTTA ATTATATCAA GAGTCATCTC TTGAGTTTCT TGGTGGTGCT CCTCGAGTAA AAGAACGGTT TTCAAACCTA CTACGGAATT CTGAATAACT
```

FIG. 9A

```
     aluI
    sstI
    sacI
    hgiJII
    hgiAI/aspHI
    ecl136II
    bsp1286
    bsiHKAI
    bmyI
    banII                                                    aluI                                                    nlaIV
                                                     sau3AI pvuII                                                 scrFI
                                                     mboI/ndeII[dam-]                                             mvaI
                                                     dpnI[dam+]                                                   ecoRII
                                                    pvuI/bspCI                                                    dsaV
                                                pleI dpnII[dam-]                                                  bstNI
                                                hinfI  taqI[dam-]                                                 apyI[dcm+]
                                            rmaI    mcrI   nspBII                                                bsaJI
   taqI                                     maeI   taqI[dam-]
  1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGAGTCGATC GACAGCTGTG GAATGTGTGT CAGTTAGGGT GTGGAAAGTC CCCAGGCTCC CCAGCAGGCA
    AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCTCAGCTAG CTGTCGACAC CTTACACACA GTCAATCCCA CACCTTTCAG GGGTCCGAGG GGTCGTCCGT

                                                                         nlaIV
               sfaNI                     scrFI        scrFI                                    sfaNI
             ppu10I                      mvaI              mvaI                                          ppu10I
             nsiI/avaIII                 ecoRII       ecoRII                                 nsiI/avaIII
            nlaIII                       dsaV         dsaV                                  nlaIII
           sphI                          bstNI             bstNI                                       sphI
           nspI                          apyI[dcm+]        apyI[dcm+]                                  nspI
           nspHI                        sexAI             bsaJI                                        nspHI
101 GAAGTATGCA AAGCATGCAT CTCAATTAGT CAGCAACCAG GTGTGGAAAG TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC ATCTCAATTA
    CTTCATACGT TTCGTACGTA GAGTTAATCA GTCGTTGGTC CACACCTTTC AGGGGTCCGA GGGGTCGTCC GTCTTCATAC GTTTCGTACG TAGAGTTAAT

                                                                                             nlaIII
                                                                                            styI
                                                                                            ncoI
                                          aciI                                         bslI dsaI
               aciI          aciI  fokI                 aciI bsrI  aciI                aciI bsaJI
201 GTCAGCAACC ATAGTCCCGC CCCTAACTCC GCCCATCCCG CCCCTAACTC CGCCCAGTTC CGCCCATTCT CCGCCCCATG GCTGACTAAT TTTTTTTATT
    CAGTCGTTGG TATCAGGGCG GGGATTGAGG CGGGTAGGGC GGGGATTGAG GCGGGTCAAG GCGGGTAAGA GGCGGGGTAC CGACTGATTA AAAAAAATAA

                                                                                   rmaI
                                                                                  styI
               fnu4HI                                                             bsaJI                      haeIII/palI
               bglI                                                               blnI                      mcrI
              sfiI                                                                avrII          aluI       eagI/xmaIII/eclXI
              haeIII/palI                                                        haeIII/palI    rmaI        eaeI
            mnlI   mnlI      ddeI                                               stuI            maeI        cfrI
       haeIII/palI bsaJI mnlI   aluI                      mnlI                  haeI            nheI       mspI
     mnlI bsaJI aciI   haeIII/palI                    mnlI                     mnlI maeI       aluI        hpaII
301 TATGCAGAGG CCGAGGCCGC CTCGGCCTCT GAGCTATTCC AGAAGTAGTG AGGAGGCTTT TTTGGAGGCC TAGGCTTTTG CAAAAAGCTA GCTTATCCGG
    ATACGTCTCC GGCTCCGGCG GAGCCGGAGA CTCGATAAGG TCTTCATCAC TCCTCCGAAA AAACCTCCGG ATCCGAAAAC GTTTTTCGAT CGAATAGGCC
```

FIG. 8

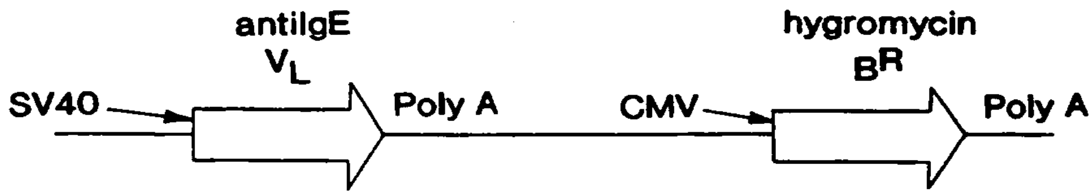
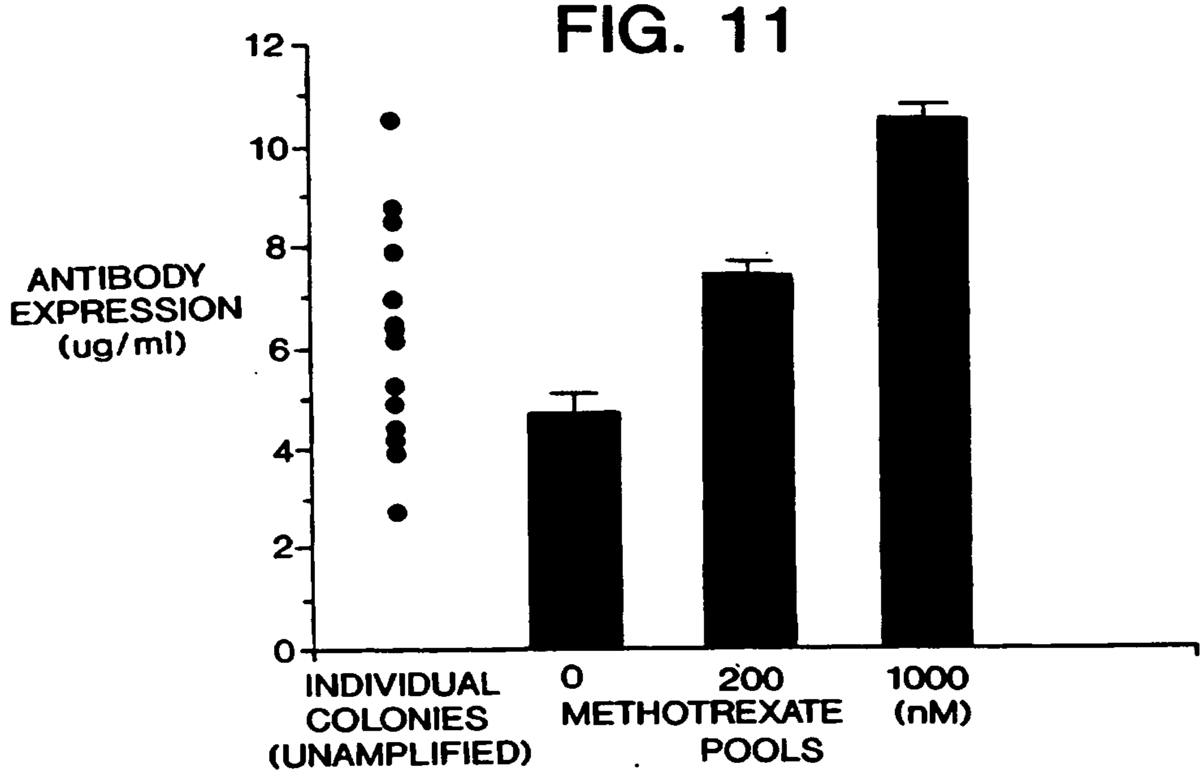
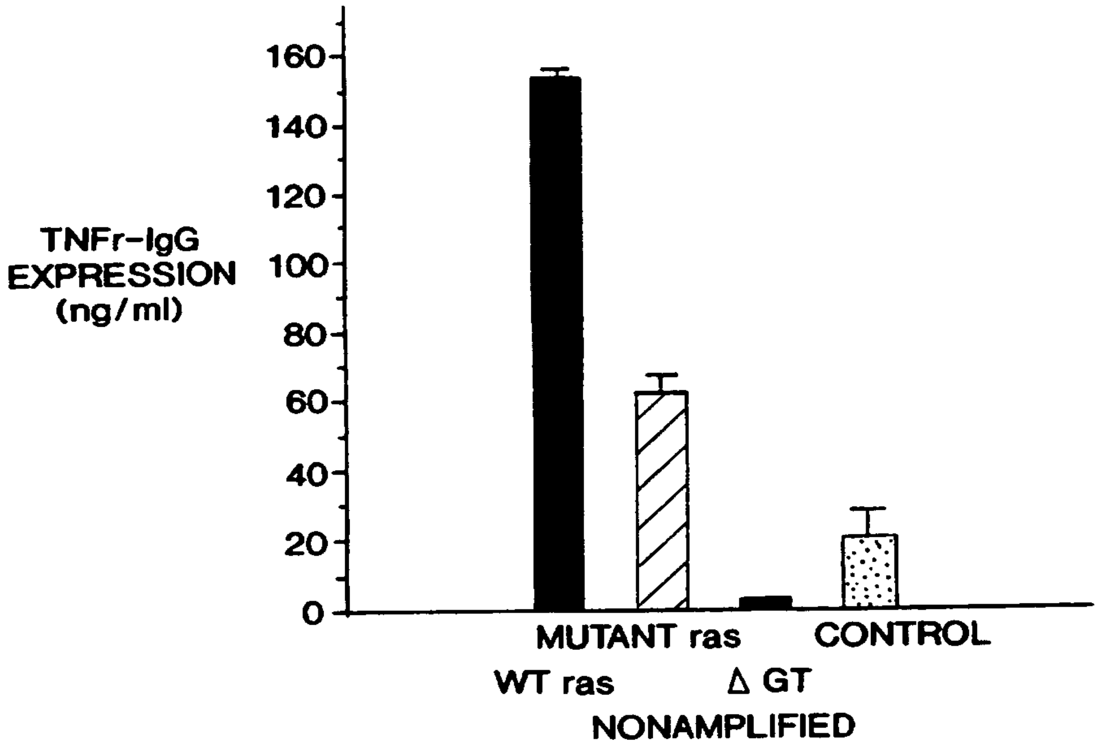

antiIgE
V_L
hygromycin
B^R
SV40
Poly A
CMV
Poly A

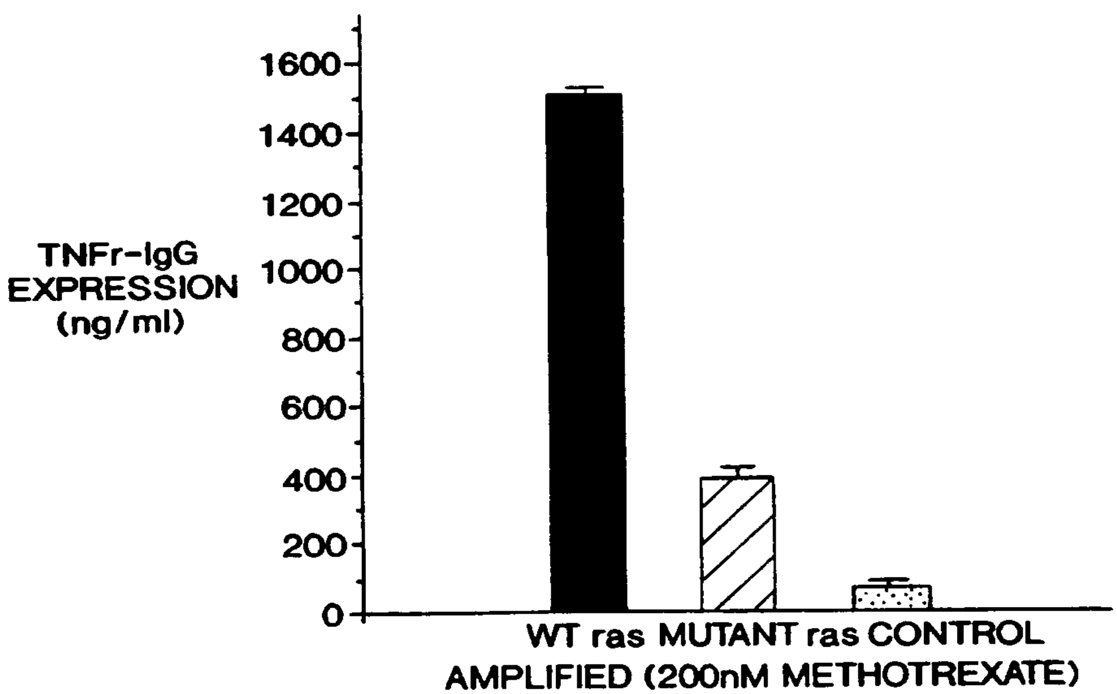
FIG. 11


ANTIBODY EXPRESSION (ug/ml)
12
10
8
6
4
2
0
INDIVIDUAL COLONIES (UNAMPLIFIED)
0
200
1000
METHOTREXATE POOLS
(nM)



160
140
120
TNFr-IgG EXPRESSION (ng/ml)
100
80
60
40
20
0
MUTANT ras
CONTROL
WT ras
Δ GT
NONAMPLIFIED

FIG. 7A



1600
1400
1200
TNFr-IgG EXPRESSION (ng/ml)
1000
800
600
400
200
0
WT ras
MUTANT ras
CONTROL
AMPLIFIED (200nM METHOTREXATE)

FIG. 7B

FIG. 6R

```
        haeIII/palI
       haeI
     scrFI
     mvaI bslI
     ecoRII                 nlaIII
     dsaV                  nspI
     bstNI        haeIII/palI  nspHI                   tfiI                                    bsrBI
     apyI[dcm+]   haeI         aflIII                  hinfI                aciI         aluI     aciI
6501 CCTGGCCTTT TGCTGGCCTT TTGCTCACAT GTTCTTTCCT GCGTTATCCC CTGATTCTGT GGATAACCGT ATTACCGCCT TTGAGTGAGC TGATACCGCT
     GGACCGGAAA ACGACCGGAA AACGAGTGTA CAAGAAAGGA CGCAATAGGG GACTAAGACA CCTATTGGCA TAATGGCGGA AACTCACTCG ACTATGGCGA

                                                                                          thaI
                                                                                          fnuDII/mvnI
                                                                                          bstUI
                                                                                          bsh1236I
                                                                                         hinPI
                                                                                         hhaI/cfoI
                                                                                        thaI
                                                                                        fnuDII/mvnI
       fnu4HI               fnu4HI                           sapI  hinPI                bstUI   haeIII/palI
       bbvI                 bbvI pleI                        mboII hhaI/cfoI            bsh1236I          tru9I
      aciI                 hinPI  hinfI                      earI/ksp632I        mnlI   bslI     eaeI  tfiI aseI/asnI/vspI
     fnu4HI      mcrI      hhaI/cfoI        mnlI    aciI     haeII          aciI        aciI     cfrI  hinfI mseI
6601 CGCCGCAGCC GAACGACCGA GCGCAGCGAG TCAGTGAGCG AGGAAGCGGA AGAGCGCCCA ATACGCAAAC CGCCTCTCCC CGCGCGTTGG CCGATTCATT
     GCGGCGTCGG CTTGCTGGCT CGCGTCGCTC AGTCACTCGC TCCTTCGCCT TCTCGCGGGT TATGCGTTTG GCGGAGAGGG GCGCGCAACC GGCTAAGTAA

                                                                                               scrFI
                                                                                               mvaI
                                                                                               ecoRII
                                                                                               dsaV
       aluI                                                       tru9I                   nlaIV bstNI
       pvuII                                        hinPI         mseI     maeIII         hgiCI apyI[dcm+]
       nspBII                 bsrI    aciI          hhaI/cfoI   aseI/asnI/vspI  mnlI       banI bsaJI
6701 AATCCAGCTG GCACGACAGG TTTCCCGACT GGAAAGCGGG CAGTGAGCGC AACGCAATTA ATGTGAGTTA CCTCACTCAT TAGGCACCCC AGGCTTTACA
     TTAGGTCGAC CGTGCTGTCC AAAGGGCTGA CCTTTCGCCC GTCACTCGCG TTGCGTTAAT TACACTCAAT GGAGTGAGTA ATCCGTGGGG TCCGAAATGT

                                                                                            tru9I
                                                                                            mseI
                                                                                           aseI/asnI/vspI
                mspI                        aciI                                          xmnI
                hpaII                      bsrBI                     aluI      nlaIII     asp700
6801 CTTTATGCTT CCGGCTCGTA TGTTGTGTGG AATTGTGAGC GGATAACAAT TTCACACAGG AAACAGCTAT GACCATGATT ACGAATTAA
     GAAATACGAA GGCCGAGCAT ACAACACACC TTAACACTCG CCTATTGTTA AAGTGTGTCC TTTGTCGATA CTGGTACTAA TGCTTAATT

>length: 6889
```

FIG. 6Q

```
                                                    scrFI                                                  aciI
                                                    nciI                                                 nspBII
                        fnu4HI                      mspI                               mspI             fnu4HI
               alwNI    bbvI                        hpaII                              hpaII            bbvI
             bsrI  fnu4HI                           dsaV       pleI                   bsaWI           hinPI mcrI
       maeIII      bbvI     bsrI                    cauII      hinfI               maeIII             hhaI/cfoI
6101 TAATCCTGTT ACCAGTGGCT GCTGCCAGTG GCGATAAGTC GTGTCTTACC GGGTTGGACT CAAGACGATA GTTACCGGAT AAGGCGCAGC GGTCGGGCTG
     ATTAGGACAA TGGTCACCGA CGACGGTCAC CGCTATTCAG CACAGAATGG CCCAACCTGA GTTCTGCTAT CAATGGCCTA TTCCGCGTCG CCAGCCCGAC

              hgiAI/aspHI
              bsp1286
              bsiHKAI
              bmyI                                                                                      hinPI
              apaLI/snoI                                                                                hhaI/cfoI
              alw44I/snoI   aluI                             ddeI       scfI                           haeII
6201 AACGGGGGGT TCGTGCACAC AGCCCAGCTT GGAGCGAACG ACCTACACCG AACTGAGATA CCTACAGCGT GAGCATTGAG AAAGCGCCAC GCTTCCCGAA
     TTGCCCCCCA AGCACGTGTG TCGGGTCGAA CCTCGCTTGC TGGATGTGGC TTGACTCTAT GGATGTCGCA CTCGTAACTC TTTCGCGGTG CGAAGGGCTT

                                                                          scrFI
                                                                          mvaI          scrFI
                                                                          ecoRII  mvaI
                         mspI                                             dsaV    ecoRII
                         hpaII                                            bstNI         dsaV
                         bslI   fnu4HI                     hinPI mnlI     bsaJI         bstNI
            aciI         bsaWI  aciI                       hhaI/cfoI  aluI apyI[dcm+]   apyI[dcm+]
6301 GGGAGAAAGG CGGACAGGTA TCCGGTAAGC GGCAGGGTCG GAACAGGAGA GCGCACGAGG GAGCTTCCAG GGGGAAACGC CTGGTATCTT TATAGTCCTG
     CCCTCTTTCC GCCTGTCCAT AGGCCATTCG CCGTCCCAGC CTTGTCCTCT CGCGTGCTCC CTCGAAGGTC CCCCTTTGCG GACCATAGAA ATATCAGGAC

                                                                                                  haeIII/palI
                                                                                                 fnu4HI
                                                                                                 aciI
                                                                                                thaI bslI
                                                                                                fnuDII/mvnI
                              taqI                                nlaIV                         bstUI
               mnlI drdI   hgaI          sfaNI                 aciI                             bsh1236I      nlaIV
6401 TCGGGTTTCG CCACCTCTGA CTTGAGCGTC GATTTTTGTG ATGCTCGTCA GGGGGGCGGA GCCTATGGAA AAACGCCAGC AACGCGGCCT TTTTACGGTT
     AGCCCAAAGC GGTGGAGACT GAACTCGCAG CTAAAAACAC TACGAGCAGT CCCCCCGCCT CGGATACCTT TTTGCGGTCG TTGCGCCGGA AAAATGCCAA
```

FIG. 6P

```
                                                                                                  rmaI      sau3AI
                                                                                              sau3AI hphI   mboI/ndeII[dam-]
                                                                                              mboI/ndeII[dam-]
                                                                                              dpnI[dam+]    dpnI[dam+]
                                                                                              dpnII[dam-)   dpnII[dam-)
                                                                 tru9I             ahaIII/draI   maeI       alwI[dam-]
                                                                 mseI          tru9I  tru9I  bstYI/xhoII  bstYI/xhoII
                 maeIII                                        ahaIII/draI     mseI   mseI   alwI[dam-] mboII[dam-]
5701 AAGCATTGGT AACTGTCAGA CCAAGTTTAC TCATATATAC TTTAGATTGA TTTAAAACTT CATTTTTAAT TTAAAAGGAT CTAGGTGAAG ATCCTTTTTG
     TTCGTAACCA TTGACAGTCT GGTTCAAATG AGTATATATG AAATCTAACT AAATTTTGAA GTAAAAATTA AATTTTCCTA GATCCACTTC TAGGAAAAAC

                                                                                           sau3AI
                                                                                           mboI/ndeII[dam-]
                                                                                           dpnI[dam+]  sau3AI
                                                                                           dpnII[dam-] mboI/ndeII[dam-]
                                                                                          bstYI/xhoII  dpnI[dam+]
                                                                                   sau3AI  alwI[dam-]  dpnII[dam-]
                nlaIII           maeII                                             mboI/ndeII[dam-]    alwI[dam-]
               rcaI            tru9I                    hgaI                       dpnI[dam+] mboII[dam-]
               bspHI           mseI                  ddeI                          dpnII[dam-]        bstYI/xhoII
5801 ATAATCTCAT GACCAAAATC CCTTAACGTG AGTTTTCGTT CCACTGAGCG TCAGACCCCG TAGAAAAGAT CAAAGGATCT TCTTGAGATC CTTTTTTTCT
     TATTAGAGTA CTGGTTTTAG GGAATTGCAC TCAAAAGCAA GGTGACTCGC AGTCTGGGGC ATCTTTTCTA GTTTCCTAGA AGAACTCTAG GAAAAAAAGA

                                                                           sau3AI
      thaI                                                                 mboI/ndeII[dam-]
      fnuDII/mvnI                                                          dpnI[dam+]
      bstUI                                                                dpnII[dam-)
      bsh1236I                                                            alwI[dam-)
     hinPI      fnu4HI                             aciI                 mspI                                    bsrI
     hhaI/cfoI  bbvI                   aciI      nspBII                 hpaII     aluI                      maeIII  eco57I
5901 GCGCGTAATC TGCTGCTTGC AAACAAAAAA ACCACCGCTA CCAGCGGTGG TTTGTTTGCC GGATCAAGAG CTACCAACTC TTTTTCCGAA GGTAACTGGC
     CGCGCATTAG ACGACGAACG TTTGTTTTTT TGGTGGCGAT GGTCGCCACC AAACAAACGG CCTAGTTCTC GATGGTTGAG AAAAAGGCTT CCATTGACCG

            hinPI                      rmaI               haeIII/palI
            hhaI/cfoI                  maeI      bslI     haeI                   scfI    aciI        mnlI
6001 TTCAGCAGAG CGCAGATACC AAATACTGTC CTTCTAGTGT AGCCGTAGTT AGGCCACCAC TTCAAGAACT CTGTAGCACC GCCTACATAC CTCGCTCTGC
     AAGTCGTCTC GCGTCTATGG TTTATGACAG GAAGATCACA TCGGCATCAA TCCGGTGGTG AAGTTCTTGA GACATCGTGG CGGATGTATG GAGCGAGACG
```

FIG. 60

```
                                                                              sau96I
                                                                              avaII
                                                                       sau3AI asuI
                                                  haeIII/palI          mboI/ndeII[dam-]
                                                 eaeI                  dpnI[dam+]
                                                 cfrI                  dpnII[dam-]
                     fnu4HI                     fnu4HI                pvuI/bspCI
                     bbvI       nlaIII          aciI                  mcrI  mnlI        aluI  aciI
5201 TAAGAGAATT ATGCAGTGCT GCCATAACCA TGAGTGATAA CACTGCGGCC AACTTACTTC TGACAACGAT CGGAGGACCG AAGGAGCTAA CCGCTTTTTT
     ATTCTCTTAA TACGTCACGA CGGTATTGGT ACTCACTATT GTGACGCCGG TTGAATGAAG ACTGTTGCTA GCCTCCTGGC TTCCTCGATT GGCGAAAAAA

                       maeIII
                    nlaIII
                 sau3AI                        mspI
                 mboI/ndeII[dam-]   sau3AI nlaIV
                 dpnI[dam+]         mboI/ndeII[dam-] aluI
                 dpnII[dam-]        dpnI[dam+] hpaII                                              fnu4HI
          nlaIII alwI[dam-]         dpnII[dam-] bsaWI                           maeIII    sfaNI  bbvI
5301 GCACAACATG GGGGATCATG TAACTCGCCT TGATCGTTGG GAACCGGAGC TGAATGAAGC CATACCAAAC GACGAGCGTG ACACCACGAT GCCAGCAGCA
     CGTGTTGTAC CCCCTAGTAC ATTGAGCGGA ACTAGCAACC CTTGGCCTCG ACTTACTTCG GTATGGTTTG CTGCTCGCAC TGTGGTGCTA CGGTCGTCGT

                                                         mspI
                  hinPI                                  hpaII
                 mstI                                   scrFI
                 aviII/fspI    bsrI                aluI nciI       tru9I     fokI                  sau96I
             maeII hhaI/cfoI tru9I               rmaI   dsaV       mseI   bsrI      aciI           avaII
            psp1406I        mseI                 maeI   cauII     aseI/asnI/vspI  mnlI             asuI
5401 ATGGCAACAA CGTTGCGCAA ACTATTAACT GGCGAACTAC TTACTCTAGC TTCCCGGCAA CAATTAATAG ACTGGATGGA GGCGGATAAA GTTGCAGGAC
     TACCGTTGTT GCAACGCGTT TGATAATTGA CCGCTTGATG AATGAGATCG AAGGGCCGTT GTTAATTATC TGACCTACCT CCGCCTATTT CAACGTCCTG

                  bglI                                    mspI             thaI
                 sau96I                                   hpaII            fnuDII/mvnI           haeIII/palI
                 haeIII/palI                             cfr10I            bstUI                sau96I
           hinPI asuI   mspI                          nlaIV hphI      bsmAI aciI      fnu4HI  nlaIV
           hhaI/cfoI    hpaII                       gsuI/bpmI        bsaI bsh1236I    bbvI bsrI asuI
5501 CACTTCTGCG CTCGGCCCTT CCGGCTGGCT GGTTTATTGC TGATAAATCT GGAGCCGGTG AGCGTGGGTC TCGCGGTATC ATTGCAGCAC TGGGGCCAGA
     GTGAAGACGC GAGCCGGGAA GGCCGACCGA CCAAATAACG ACTATTTAGA CCTCGGCCAC TCGCACCCAG AGCGCCATAG TAACGTCGTG ACCCCGGTCT

                                                                                 ddeI
                                                                            sau3AI       nlaIV
                                           pleI                             mboI/ndeII[dam-] mnlI
                                           hinfI                            dpnI[dam+]   hgiCI        tru9I
            mnlI                     eam1105I             fokI              dpnII[dam-]  banI         mseI
5601 TGGTAAGCCC TCCCGTATCG TAGTTATCTA CACGACGGGG AGTCAGGCAA CTATGGATGA ACGAAATAGA CAGATCGCTG AGATAGGTGC CTCACTGATT
     ACCATTCGGG AGGGCATAGC ATCAATAGAT GTGCTGCCCC TCAGTCCGTT GATACCTACT TGCTTTATCT GTCTAGCGAC TCTATCCACG GAGTGACTAA
```

FIG. 6N

```
                                                          nlaIV
                                                        aciI
                                                       thaI
                                                       fnuDII/mvnI
                     hinlI/acyI                        bstUI                                                     rcaI
                     ahaII/bsaHI                       bsh1236I                                                  bspHI
                     aatII                            hinPI                                                  bsrBI
                 ddeI maeII                           hhaI/cfoI                                              aciI nlaIII
4701 AATAATGGTT TCTTAGACGT CAGGTGGCAC TTTTCGGGGA AATGTGCGCG GAACCCCTAT TTGTTTATTT TTCTAAATAC ATTCAAATAT GTATCCGCTC
     TTATTACCAA AGAATCTGCA GTCCACCGTG AAAAGCCCCT TTACACGCGC CTTGGGGATA AACAAATAAA AAGATTTATG TAAGTTTATA CATAGGCGAG

                                                     mboII
       bsmAI                           sspI          earI/ksp632I                                           fnu4HI
                                                                                                            aciI
4801 ATGAGACAAT AACCCTGATA AATGCTTCAA TAATATTGAA AAAGGAAGAG TATGAGTATT CAACATTTCC GTGTCGCCCT TATTCCCTTT TTTGCGGCAT
     TACTCTGTTA TTGGGACTAT TTACGAAGTT ATTATAACTT TTTCCTTCTC ATACTCATAA GTTGTAAAGG CACAGCGGGA ATAAGGGAAA AAACGCCGTA

                                                                             hgiAI/aspHI
                                                                             bsp1286
                                                                  sau3AI     bsiHKAI                      sau3AI
                                                                  mboI/ndeII[dam-]                        mboI/ndeII[dam-]
                                                                  dpnI[dam+] bmyI                         dpnI[dam+]
                                                                  dpnII[dam-]                            bstYI/xhoII
                                                             eco57I          apaLI/snoI               bsrI dpnII[dam-]
                         hphI             hphI          sfaNI  mboII[dam-]   alw44I/snoI  maeIII taqI    alwI[dam-]
4901 TTTGCCTTCC TGTTTTTGCT CACCCAGAAA CGCTGGTGAA AGTAAAAGAT GCTGAAGATC AGTTGGGTGC ACGAGTGGGT TACATCGAAC TGGATCTCAA
     AAACGGAAGG ACAAAAAACGA GTGGGTCTTT GCGACCACTT TCATTTTCTA CGACTTCTAG TCAACCCACG TGCTCACCCA ATGTAGCTTG ACCTAGAGTT

                                                                                           aciI
              sau3AI                                                                      thaI
              mboI/ndeII[dam-]             maeII                                          fnuDII/mvnI
              dpnI[dam+]                  psp1406I            hgiAI/aspHI                 bstUI
              dpnII[dam-]                xmnI                 bsp1286 tru9I               bsh1236I                 hinlI/acyI
       aciI   alwI[dam-]                 asp700               bsiHKAI mseI               hinPI                     hgaI
     nspBII  bstYI/xhoII              mboII                   bmyI    ahaIII/draI        hhaI/cfoI                 ahaII/bsaHI
5001 CAGCGGTAAG ATCCTTGAGA GTTTTCGCCC CGAAGAACGT TTTCCAATGA TGAGCACTTT TAAAGTTCTG CTATGTGGCG CGGTATTATC CCGTGATGAC
     GTCGCCATTC TAGGAACTCT CAAAAGCGGG GCTTCTTGCA AAAGGTTACT ACTCGTGAAA ATTTCAAGAC GATACACCGC GCCATAATAG GGCACTACTG

     scrFI
     nciI
     mspI
     hpaII                                                        rsaI
     dsaV                    aciI                                 csp6I   bsrI
     cauII      bcgI  mcrI  fnu4HI           ddeI                scaI  hphI  maeIII      sfaNI     fokI   nlaIII
5101 GCCGGGCAAG AGCAACTCGG TCGCCGCATA CACTATTCTC AGAATGACTT GGTTGAGTAC TCACCAGTCA CAGAAAAGCA TCTTACGGAT GGCATGACAG
     CGGCCCGTTC TCGTTGAGCC AGCGGCGTAT GTGATAAGAG TCTTACTGAA CCAACTCATG AGTGGTCAGT GTCTTTTCGT AGAATGCCTA CCGTACTGTC
```

FIG. 6M

```
                           maeII pleI          tru9I    pleI                        bslI
                           drdI   hinfI  maeII  mseI     hinfI        bsrI           bslI  avaI
4201 ATCGCCCTGA TAGACGGTTT TTCGCCCTTT GACGTTGGAG TCCACGTTCT TTAATAGTGG ACTCTTGTTC CAAACTGGAA CAACACTCAA CCCTATCTCG
     TAGCGGGACT ATCTGCCAAA AAGCGGGAAA CTGCAACCTC AGGTGCAAGA AATTATCACC TGAGAACAAG GTTTGACCTT GTTGTGAGTT GGGATAGAGC

                                                                                          thaI
                                                                                          fnuDII/mvnI
                                                         tru9I                   tru9I  apoI tru9I     tru9I
                                                         mseI            tru9I   mseI bstUI  mseI       mseI
                                                haeIII/palI      aluI    mseI   apoI  bsh1236I      sspI
4301 GGCTATTCTT TTGATTTATA AGGGATTTTG CCGATTTCGG CCTATTGGTT AAAAAATGAG CTGATTTAAC AAAAATTTAA CGCGAATTTT AACAAAATAT
     CCGATAAGAA AACTAAATAT TCCCTAAAAC GGCTAAAGCC GGATAACCAA TTTTTTACTC GACTAAATTG TTTTTAAATT GCGCTTAAAA TTGTTTTATA

                      hgiAI/aspHI
                      bsp1286
                      bsiHKAI                                                                         hinPI
                      bmyI    ddeI                aciI                            maeIII         fnu4HI
  maeII               apaLI/snoI  rsaI           fnu4HI    tru9I              maeII bsrI      nlaIII hhaI/cfoI
  psp1406I            alw44I/snoI csp6I        sfaNI      mseI        aciI   bsaAI tth111I/aspI bbvI
4401 TAACGTTTAC AATTTTATGG TGCACTCTCA GTACAATCTG CTCTGATGCC GCATAGTTAA GCCAACTCCG CTATCGCTAC GTGACTGGGT CATGGCTGCG
     ATTGCAAATG TTAAAATACC ACGTGAGAGT CATGTTAGAC GAGACTACGG CGTATCAATT CGGTTGAGGC GATAGCGATG CACTGACCCA GTACCGACGC

                                                                                            scrFI
                             hinPI                     sfaNI                                nciI
                             hhaI/cfoI               mspI                                   mspI
                            thaI                     hpaII                                  hpaII    nspI
                            fnuDII/mvnI             scrFI                                   dsaV     nspHI
                            bstUI                   nciI                               esp3I       fnu4HI
                     nspBII bsh1236I                dsaV fokI                     maeIII bsmAI     bbvI
          aciI       aciI  hgaI      drdI          cauII  aciI          aluI     bslI   cauII aluI nlaIII
4501 CCCCGACACC CGCCAACACC CGCTGACGCG CCCTGACGGG CTTGTCTGCT CCCGGCATCC GCTTACAGAC AAGCTGTGAC CGTCTCCGGG AGCTGCATGT
     GGGGCTGTGG GCGGTTGTGG GCGACTGCGC GGGACTGCCC GAACAGACGA GGGCCGTAGG CGAATGTCTG TTCGACACTG GCAGAGGCCC TCGACGTACA

                                 thaI
                                 fnuDII/mvnI
                                 bstUI
                                 bsh1236I
                                hinPI
                                hhaI/cfoI                         mnlI
                               thaI                             haeIII/palI
                               fnuDII/mvnI        mboII         sau96I                                nlaIII
                               bstUI mnlI         bpuAI         asuI                           tru9I  rcaI
   mnlI     hphI       hphI    bsh1236I           bbsI         eco0109I/draII                 mseI   bspHI
4601 GTCAGAGGTT TTCACCGTCA TCACCGAAAC GCGCGAGGCA GTATTCTTGA AGACGAAAGG GCCTCGTGAT ACGCCTATTT TTATAGGTTA ATGTCATGAT
     CAGTCTCCAA AAGTGGCAGT AGTGGCTTTG CGCGCTCCGT CATAAGAACT TCTGCTTTCC CGGAGCACTA TGCGGATAAA AATATCCAAT TACAGTACTA
```

FIG. 6L

```
                                                                                                   hinPI
                                                                                                   hhaI/cfoI
                                                                                                  nlaIV
                                                      sau3AI                                      narI
                                           sau96I     mboI/ndeII[dam-]                            kasI
                                           haeIII/palI                                            hinlI/acyI
                                           asuI       dpnI[dam+]                                  hgiCI
                     aluI                mnlI         dpnII[dam-]                                 haeII      aciI
                    pvuII             mboII   aciI   pvuI/bspCI                                   banI    sfaNI
     fokI           nspBII            earI/ksp632I   mcrI                        bglI             ahaII/bsaHI
3801 CATCCCCCCT TCGCCAGCTG GCGTAATAGC GAAGAGGCCC GCACCGATCG CCCTTCCCAA CAGTTGCGTA GCCTGAATGG CGAATGGCGC CTGATGCGGT
     GTAGGGGGGA AGCGGTCGAC CGCATTATCG CTTCTCCGGG CGTGGCTAGC GGGAAGGGTT GTCAACGCAT CGGACTTACC GCTTACCGCG GACTACGCCA

                                                                                                     aciI
                                                                                                  fnu4HI
                                                                        hinPI                     aciI
                                                                       thaI                      thaI
                                                                       fnuDII/mvnI               fnuDII/mvnI
                                                                       bstUI scfI     hinPI      bstUI
                                                                       bsh1236I       hhaI/cfoI hinPI
                                                                   rsaI hhaI/cfoI  fnu4HI  tru9I bsh1236I
                   sfaNI    aciI          aciI   maeII             csp6I   bslI    aciI    mseI hhaI/cfoI
3901 ATTTTCTCCT TACGCATCTG TGCGGTATTT CACACCGCAT ACGTCAAAGC AACCATAGTA CGCGCCCTGT AGCGGCGCAT TAAGCGCGGC GGGTGTGGTG
     TAAAAGAGGA ATGCGTAGAC ACGCCATAAA GTGTGGCGTA TGCAGTTTCG TTGGTATCAT GCGCGGGACA TCGCCGCGTA ATTCGCGCCG CCCACACCAC

            fnu4HI
          hinPI
          hhaI/cfoI                     hinPI
         thaI                           hhaI/cfoI
         fnuDII/mvnI                 rmaI                                                        mspI
         bstUI                     hinPI  haeII                                                  hpaII
         bsh1236I     aciI         hhaI/cfoI    bsrBI                                           naeI
     maeIII bbvI  maeIII          haeII maeI    aciI             mboII                  maeII   cfr10I
4001 GTTACGCGCA GCGTGACCGC TACACTTGCC AGCGCCCTAG CGCCCGCTCC TTTCGCTTTC TTCCCTTCCT TTCTCGCCAC GTTCGCCGGC TTTCCCCGTC
     CAATGCGCGT CGCACTGGCG ATGTGAACGG TCGCGGGATC GCGGGCGAGG AAAGCGAAAG AAGGGAAGGA AAGAGCGGTG CAAGCGGCCG AAAGGGGCAG

                    nlaIV
                   hgiJII
                   bsp1286                              nlaIV                                   maeII     haeIII/palI
                   bmyI                                 hgiCI  taqI                            draIII    sau96I
     aluI          banII         nlaIV                  banI mnlI                     hphI     bsaAI     asuI
4101 AAGCTCTAAA TCGGGGGCTC CCTTTAGGGT TCCGATTTAG TGCTTTACGG CACCTCGACC CCAAAAAACT TGATTTGGGT GATGGTTCAC GTAGTGGGCC
     TTCGAGATTT AGCCCCCGAG GGAAATCCCA AGGCTAAATC ACGAAATGCC GTGGAGCTGG GGTTTTTTGA ACTAAACCCA CTACCAAGTG CATCACCCGG
```

FIG. 6K

```
           nlaIV                                                                    nlaIV
        scrFI                                          scrFI       scrFI
        mvaI                     ppu10I                     mvaI                 mvaI
        ecoRII                nsiI/avaIII              ecoRII      ecoRII
        dsaV                 nlaIII                    dsaV        dsaV
        bstNI                   sphI                        bstNI                bstNI
        apyI[dcm+]              nspI sfaNI                  apyI[dcm+]           apyI[dcm+]
       bsaJI                    nspHI                      sexAI               bsaJI
3401 GAAAGTCCCC AGGCTCCCCA GCAGGCAGAA GTATGCAAAG CATGCATCTC AATTAGTCAG CAACCAGGTG TGGAAAGTCC CCAGGCTCCC CAGCAGGCAG
     CTTTCAGGGG TCCGAGGGGT CGTCCGTCTT CATACGTTTC GTACGTAGAG TTAATCAGTC GTTGGTCCAC ACCTTTCAGG GGTCCGAGGG GTCGTCCGTC

              sfaNI
            ppu10I
            nsiI/avaIII
           nlaIII
          sphI
          nspI                                           aciI                                        bslI
          nspHI                       aciI        aciI  fokI             aciI bsrI  aciI             aciI
3501 AAGTATGCAA AGCATGCATC TCAATTAGTC AGCAACCATA GTCCCGCCCC TAACTCCGCC CATCCCGCCC CTAACTCCGC CCAGTTCCGC CCATTCTCCG
     TTCATACGTT TCGTACGTAG AGTTAATCAG TCGTTGGTAT CAGGGCGGGG ATTGAGGCGG GTAGGGCGGG GATTGAGGCG GGTCAAGGCG GGTAAGAGGC

                                                                                                      rmaI
                                                                                                     styI
                                                                                                     bsaJI
                                                 fnu4HI                                              blnI
       nlaIII                                   sfiI mnlI                                            avrII
      styI                                      haeIII/palI                                        haeIII/palI
      ncoI                                 bsaJI bglI        ddeI                                 stuI
      dsaI                               haeIII/palI bsaJI  mnlI  aluI                mnlI         haeI
      bsaJI                            mnlI  mnlI aciI    haeIII/palI              mnlI            mnlI maeI
3601 CCCCATGGCT GACTAATTTT TTTTATTTAT GCAGAGGCCG AGGCCGCCTC GGCCTCTGAG CTATTCCAGA AGTAGTGAGG AGGCTTTTTT GGAGGCCTAG
     GGGGTACCGA CTGATTAAAA AAAATAAATA CGTCTCCGGC TCCGGCGGAG CCGGAGACTC GATAAGGTCT TCATCACTCC TCCGAAAAAA CCTCCGGATC

                                                                          scrFI
                                                                          mvaI
                                                                          ecoRII
                tru9I                  haeIII/palI                        dsaV
               hpaI                   eaeI                                bstNI
               hincII/hindII          cfrI                 bsrI           apyI[dcm+]      tru9I      fnu4HI
            aluI mseI  aluI          bsrI            maeII maeIII        bsaJI   maeIII   mseI       bbvI
3701 GCTTTTGCAA AAAGCTGTTA ACAGCTTGGC ACTGGCCGTC GTTTTACAAC GTCGTGACTG GGAAAACCCT GGCGTTACCC AACTTAATCG CCTTGCAGCA
     CGAAAACGTT TTTCGACAAT TGTCGAACCG TGACCGGCAG CAAAATGTTG CAGCACTGAC CCTTTTGGGA CCGCAATGGG TTGAATTAGC GGAACGTCGT
```

FIG. 6J

```
           rmaI
         mnlI
       sau3AI
       mboI/ndeII[dam-]                        styI
       dpnI[dam+]                            aciI
       dpnII[dam-]  taqI                    fnu4HI   sau96I
       alwI[dam-]   salI    scfI           sfiI ncoI haeIII/palI
      nlaIV  maeI   hincII/hindII          haeIII/palI
      bstYI/xhoII   accI    pstI          eaeI  dsaI asuI                 aluI
      bamHI xbaI pleI      bsgI    aluI cfrI  bsaJI                    fnu4HI
      alwI[dam-] hinfI bspMI   hindIII bglI nlaIII                     bbvI           maeIII            sfaNI   apoI
3101 GGGGATCCTC TAGAGTCGAC CTGCAGAAGC TTGGCCGCCA TGGCCCAACT TGTTTATTGC AGCTTATAAT GGTTACAAAT AAAGCAATAG CATCACAAAT
     CCCCTAGGAG ATCTCAGCTG GACGTCTTCG AACCGGCGGT ACCGGGTTGA ACAAATAACG TCGAATATTA CCAATGTTTA TTTCGTTATC GTAGTGTTTA

                                                                                              sau3AI
                                                                                              mboI/ndeII[dam-]
                                                                                              dpnI[dam+]
                                                                                              dpnII[dam-]
                                                                                             pvuI/bspCI
                                                                                             mcrI
                                                                                           taqI[dam-]   tru9I
                                                                                          claI/bsp106[dam-]
                                                                                         sau3AI         mseI
                                                                                         mboI/ndeII[dam-]
                                                                                         dpnI[dam+] xmnI        hinPI
                                            rmaI                                         dpnII[dam-]  aseI/asnI/vspI
                                  bsmI   maeI                                   nlaIII  alwI[dam-]  asp700      hhaI/cfoI
3201 TTCACAAATA AAGCATTTTT TTCACTGCAT TCTAGTTGTG GTTTGTCCAA ACTCATCAAT GTATCTTATC ATGTCTGGAT CGATCGGGAA TTAATTCGGC
     AAGTGTTTAT TTCGTAAAAA AAGTGACGTA AGATCAACAC CAAACAGGTT TGAGTAGTTA CATAGAATAG TACAGACCTA GCTAGCCCTT AATTAAGCCG

                                                       rsaI
                                                       csp6I
          haeI                                        nlaIV
        styI                                          kpnI
        ncoI                                          hgiCI
        dsaI haeIII/palI                              banI                   aluI
     fnu4HI nlaIII                                    asp718     mnlI       pvuII
     bbvI   bsaJI          mnlI         mnlI          acc65I   ddeI aciI    nspBII
3301 GCAGCACCAT GGCCTGAAAT AACCTCTGAA AGAGGAACTT GGTTAGGTAC CTTCTGAGGC GGAAAGAACC AGCTGTGGAA TGTGTGTCAG TTAGGGTGTG
     CGTCGTGGTA CCGGACTTTA TTGGAGACTT TCTCCTTGAA CCAATCCATG GAAGACTCCG CCTTTCTTGG TCGACACCTT ACACACAGTC AATCCCACAC
```

FIG. 6I

```
                                                                                                    rsaI
                                                                                                    csp6I
                 bsmAI                                                                             bsp1407I    bslI
                bsaI            mnlI           taqI                                 avaI
2701 ACAAGTGCAA GGTCTCCAAC AAAGCCCTCC CAGCCCCCAT CGAGAAAACC ATCTCCAAAA CCAAAGGACA GCCCCGAGAA CCACAGGTGT ACACCCTGCC
     TGTTCACGTT CCAGAGGTTG TTTCGGGAGG GTCGGGGGTA GCTCTTTTGG TAGAGGTTTT GGTTTCCTGT CGGGGCTCTT GGTGTCCACA TGTGGGACGG

           scrFI
           nciI
           mspI
           hpaII
           dsaV
           cauII
          xmaI/pspAI
          smaI
          scrFI                   scrFI         scrFI
          nciI                    mvaI                mvaI
          dsaV                    ecoRII        ecoRII
          cauII                   dsaV          dsaV                                        aciI
          bsaJI                   bstNI               bstNI                         dsaI             nspBII
       fokI                       apyI[dcm+]          apyI[dcm+]                    bslI            fnu4HI fnu4HI
     bslI avaI  mnlI             sexAI          bspMI                               bsaJI           bbvI   bbvI
2801 CCCATCCCGG GAGGAGATGA CCAAGAACCA GGTCAGCCTG ACCTGCCTGG TCAAAGGCTT CTACCCCAGC GACATCGCCG TGGAGTGGGA GAGCAGCGGG
     GGGTAGGGCC CTCCTCTACT GGTTCTTGGT CCAGTCGGAC TGGACGGACC AGTTTCCGAA GATGGGGTCG CTGTAGCGGC ACCTCACCCT CTCGTCGCCC

                                                                                   dsaI
        mspI                                   pleI                 mnlI       hphI                   fnu4HI
        hpaII                     mnlI  nlaIII hinfI     nlaIV   mboII   scfI    aluI bsaJI       bspMI  bbvI
2901 CAGCCGGAGA ACAACTACAA CACCACGCCT CCCATGCTGG ACTCCGACGG CTCCTTCTTC CTCTACAGCA AGCTCACCGT GGACAAGAGC AGGTGGCAGC
     GTCGGCCTCT TGTTGATGTT GTGGTGCGGA GGGTACGACC TGAGGCTGCC GAGGAAGAAG GAGATGTCGT TCGAGTGGCA CCTGTTCTCG TCCACCGTCG

                                                                                                          scrFI
                                                                                                          nciI
                                                                                                          mspI
                                                                                                          hpaII
                                                                                                          dsaV
                                                                                    scrFI                 cauII
                                                                                    nciI                  bsaJI
                                                                                    mspI                haeIII/palI
                                   nlaIII                                           hpaII              mcrI
                                 ppu10I                             sapI            dsaV               eagI/xmaIII/eclXI
         xmnI mboII              nsiI/avaIII                        mboII  mnlI   bsmAI                eaeI
         asp700     nlaIII      sfaNI   mnlI           aciI         earI/ksp632I bslI  cauII           cfrI
3001 AGGGGAACAT CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCGC TTCACGCAGA AGAGCCTCTC CCTGTCTCCG GGTAAATGAG TGCGACGGCC
     TCCCCTTGTA GAAGAGTACG AGGCACTACG TACTCCGAGA CGTGTTGGCG AAGTGCGTCT TCTCGGAGAG GGACAGAGGC CCATTTACTC ACGCTGCCGG
```

FIG. 6H

```
                                                                                   eam1105I
                                                                                  sau96I
                                                                         scrFI
                                                    bsp1286              mvaI    avaII
                                                   nlaIV                 ecoRII
                                                   hgiCI                 dsaV
                                               dsaI bmyI                 bstNI   asuI        mboII mboII
                                               bsaJI                     bsaJI mnlI         bpuAI earI/ksp632I      styI
                    maeIII    mnlI    nlaIII    banI    alwNI            apyI[dcm+]         bbsI mnlI               bsaJI
2401 CTTGTGACAC ACCTCCCCCA TGCCCACGGT GCCCAGCACC TGAACTCCTG GGAGGACCGT CAGTCTTCCT CTTCCCCCCA AAACCCAAGG ATACCCTTAT
     GAACACTGTG TGGAGGGGGT ACGGGTGCCA CGGGTCGTGG ACTTGAGGAC CCTCCTGGCA GTCAGAAGGA GAAGGGGGGT TTTGGGTTCC TATGGGAATA

                         sau96I
                         nlaIV
                         avaII
                         asuI               maeII
                       mspI                pmlI
                       hpaII               eco72I                                              sau96I
                      scrFI       mnlI     bsaAI                                      drdI     avaII               maeII
                      nciI      ddeI maeIII                                        mboII     mnlI                 bsaAI
                      dsaV      eco81I     bbrPI                                   bpuAI    bsaJI    bsrI         rsaI
                      cauII    bsu36I/mstII/sauI            maeII                  bbsI    avaI   asuI            csp6I          mnlI
2501 GATTTCCCGG ACCCCTGAGG TCACGTGCGT GGTGGTGGAC GTGAGCCACG AAGACCCCGA GGTCCAGTTC AAGTGGTACG TGGACGGCGT GGAGGTGCAT
     CTAAAGGGCC TGGGGACTCC AGTGCACGCA CCACCACCTG CACTCGGTGC TTCTGGGGCT CCAGGTCAAG TTCACCATGC ACCTGCCGCA CCTCCACGTA

                                    aciI
                                   thaI
                                   fnuDII/mvnI
                                   bstUI
                                   bsh1236I
                                  sacII/sstII
                                  nspBII                                                                  scrFI
                                  kspI                                                                    mvaI bsrI
                                  dsaI                                                                    ecoRII
                                  bsaJI                                                                   dsaV
                                  aciI                                                 mnlI      econI    bstNI                rsaI
                                 fnu4HI   mnlI              maeII               hgaI     hphI      bslI     apyI[dcm+]          csp6I
2601 AATGCCAAGA CAAAGCCGCG GGAGGAGCAG TTCAACAGCA CGTTCCGTGT GGTCAGCGTC CTCACCGTCC TGCACCAGGA CTGGCTGAAC GGCAAGGAGT
     TTACGGTTCT GTTTCGGCGC CCTCCTCGTC AAGTTGTCGT GCAAGGCACA CCAGTCGCAG GAGTGGCAGG ACGTGGTCCT GACCGACTTG CCGTTCCTCA
```

FIG. 6G

```
                                                                           hgiAI/aspHI                           hgiAI/aspHI
                                                                           bsp1286        scrFI            bsp1286
                                                                           bsiHKAI        mvaI                   bsiHKAI
                                                     mnlI            sau96I               ecoRII           bmyI
                                                  alwNI              avaII bmyI mnlI      dsaV             apaLI/snoI
                                                 fnu4HI              asuI  apaLI/snoI     bstNI                  alw44I/snoI
                                 bsrI      munI  bbvI       mnlI    nlaIV  alw44I/snoI    apyI[dcm+]       draIII
2001 CGGCATTATT GGAGTGAAAA CCTTTTCCAG TGCTTCAATT GCAGCCTCTG CCTCAATGGG ACCGTGCACC TCTCCTGCCA GGAGAAACAG AACACCGTGT
     GCCGTAATAA CCTCACTTTT GGAAAAGGTC ACGAAGTTAA CGTCGGAGAC GGAGTTACCC TGGCACGTGG AGAGGACGGT CCTCTTTGTC TTGTGGCACA

                                                                                      hgiAI/aspHI
                                                                                      bsp1286
                                                                                      bsiHKAI
                                                                                      bmyI
                                                                                gsuI/bpmI
                                                                               scrFI
                                                                               mvaI   apaLI/snoI
                                                                               ecoRII
                                                                               dsaV
                 bspMI                                 scfI                    bstNI  alw44I/snoI
       bspMI nlaIII          ddeI                 bsmAI      maeIII            apyI[dcm+]
2101 GCACCTGCCA TGCAGGTTTC TTTCTAAGAG AAAACGAGTG TGTCTCCTGT AGTAACTGTA AGAAAAGCCT GGAGTGCACG AAGTTGTGCC TACCCCAGAT
     CGTGGACGGT ACGTCCAAAG AAAGATTCTC TTTTGCTCAC ACAGAGGACA TCATTGACAT TCTTTTCGGA CCTCACGTGC TTCAACACGG ATGGGGTCTA

                                                             aluI
                                                           sstI
                                                           sacI
                                                           hgiJII
                                                           hgiAI/aspHI
                                                           ecl136II                                                bsp1286
                                    nlaIV                  bsp1286                                                nlaIV
                  bsp1286    pleI   hgiCI                  bsiHKAI                maeIII          nlaIII          hgiCI
             tru9I       mnlI  ddeI                        bmyI                  hphI            nspI  dsaI  banI
             mseI bmyI ddeI  hinfI  banI                   banII          draIII                 nspHI bsaJI bmyI
2201 TGAGAATGTT AAGGGCACTG AGGACTCAGG CACCACAGAC AAGAGAGTTG AGCTCAAAAC CCCACTTGGT GACACAACTC ACACATGCCC ACGGTGCCCA
     ACTCTTACAA TTCCCGTGAC TCCTGAGTCC GTGGTGTCTG TTCTCTCAAC TCGAGTTTTG GGGTGAACCA CTGTGTTGAG TGTGTACGGG TGCCACGGGT

                                              bsp1286
                                             nlaIV                                                bsp1286
     hgiJII                                  hgiCI    hgiJII                                     nlaIV   hgiJII
     bsp1286                             dsaI bmyI    bsp1286                               dsaI hgiCI   bsp1286
     bmyI                           bsp1286  banI     bmyI                                  bsaJI bmyI   bmyI
     banII        maeIII    mnlI    bmyI bsaJI        banII         maeIII   mnlI    nlaIII      banI    banII
2301 GAGCCCAAAT CTTGTGACAC ACCTCCCCCG TGCCCACGGT GCCCAGAGCC CAAATCTTGT GACACACCTC CCCCATGCCC ACGGTGCCCA GAGCCCAAAT
     CTCGGGTTTA GAACACTGTG TGGAGGGGGC ACGGGTGCCA CGGGTCTCGG GTTTAGAACA CTGTGTGGAG GGGGTACGGG TGCCACGGGT CTCGGGTTTA
```

FIG. 6F

```
                                 scrFI
                                 nciI
                                 mspI
                                 hpaII
                                 dsaV
                                 cauII
                                xmaI/pspAI
                                smaI
                                scrFI
                                nciI
                                dsaV
                                cauII
                                bslI
                              sau96I
                              haeIII/palI
                              asuI
                           scrFI
                           mvaI bsaJI
                           ecoRII
                           dsaV                                    nlaIV
            rsaI           bstNI bsaJI              scfI               fnu4HI                      alwNI
            csp6I          bslI avaI                pstI               aciI         aciI              ddeI
           bsp1407I        apyI[dcm+]               bsgI            bsrBI       hphI   eco57I        mnlI
1801 CCTACTTGTA CAATGACTGT CCAGGCCCGG GGCAGGATAC GGACTGCAGG GAGTGTGAGA GCGGCTCCTT CACCGCTTCA GAAAACCACC TCAGACACTG
     GGATGAACAT GTTACTGACA GGTCCGGGCC CCGTCCTATG CCTGACGTCC CTCACACTCT CGCCGAGGAA GTGGCGAAGT CTTTTGGTGG AGTCTGTGAC

                                                                          scrFI
                                                       mboII              nciI
                                                      earI/ksp632I        mspI
          fnu4HI                                   sau3AI                 hpaII
         aluI                                      mboI/ndeII[dam-]       dsaV                scfI              mspI
        pvuII                                      dpnI[dam+]             cauII               pstI              hpaII
        nspBII                                     dpnII[dam-]         sau96I                 bsgI           rsaI
      ddeI                                        bstYI/xhoII          avaII                 fnu4HI          csp6I
     mnlI bbvI                                    bglII                asuI     draIII       bbvI  mboII   bsrI cfr10I
1901 CCTCAGCTGC TCCAAATGCC GAAAGGAAAT GGGTCAGGTG GAGATCTCTT CTTGCACAGT GGACCGGGAC ACCGTGTGTG GCTGCAGGAA GAACCAGTAC
     GGAGTCGACG AGGTTTACGG CTTTCCTTTA CCCAGTCCAC CTCTAGAGAA GAACGTGTCA CCTGGCCCTG TGGCACACAC CGACGTCCTT CTTGGTCATG
```

FIG. 6E

```
                                                                                                    haeIII/palI
                                                                                                   eaeI
                                                        scrFI                                cfrI
                                                        mvaI                                      mspI
                                                        ecoRII                              hpaII
                                                        dsaV                               scrFI
                                                        bstNI                                    nciI
                                                        apyI[dcm+]      mnlI              ecoRI  dsaV
                                                      gsuI/bpmI         bsaJI      taqI  apoI   cauII
       scfI        fokI                           maeIII     hincII/hindII         claI/bsp106  bsaJI      aluI
1501 CACTATAGAA TAACATCCAC TTTGCCTTTC TCTCCACAGG TGTCACTCCA GGTCAACTGC ACCTCGGTTC TATCGATTGA ATTCCCCGGC CATAGCTGTC
     GTGATATCTT ATTGTAGGTG AAACGGAAAG AGAGGTGTCC ACAGTGAGGT CCAGTTGACG TGGAGCCAAG ATAGCTAACT TAAGGGGCCG GTATCGACAG

                                                          gsuI/bpmI
                                                         scrFI
                                                         mvaI
                                                         ecoRII                                          mnlI
                                                         dsaV                                            ecoNI
              mnlI                           nspBII      bstNI                         ddeI                sau96I
            haeIII/palI                  fnu4HI     hgiAI/aspHI                       mnlI                 nlaIV
           sau96I                     fnu4HI aciI   bsp1286                           eco81I               avaII
           asuI                       bbvI  fnu4HI  bsiHKAI    aluI                   bsu36I/mstII/sauI    asuI  hphI
        nlaIII                   bspMI   bbvI       bmyI apyI[dcm+]                 bslI                bsrI   bslI
1601 TGGCATGGGC CTCTCCACCG TGCCTGACCT GCTGCTGCCG CTGGTGCTCC TGGAGCTGTT GGTGGGAATA TACCCCTCAG GGGTTATTGG ACTGGTCCCT
     ACCGTACCCG GAGAGGTGGC ACGGACTGGA CGACGACGGC GACCACGAGG ACCTCGACAA CCACCCTTAT ATGGGGAGTC CCCAATAACC TGACCAGGGA

        rmaI
        maeI
       styI
       bsaJI
       blnI            mboII                  styI                                         rsaI
       avrII           earI/ksp632I           bsaJI                mnlI         taqI       csp6I          nlaIV
1701 CACCTAGGGG ACAGGGAGAA GAGAGATAGT GTGTGTCCCC AAGGAAAATA TATCCACCCT CAAAATAATT CGATTTGCTG TACCAAGTGC CACAAAGGAA
     GTGGATCCCC TGTCCCTCTT CTCTCTATCA CACACAGGGG TTCCTTTTAT ATAGGTGGGA GTTTTATTAA GCTAAACGAC ATGGTTCACG GTGTTTCCTT
```

FIG. 6D

```
                                                                               haeIII/palI
                                                                              haeI
                                                       scrFI          scrFI                        nlaIII
                                                       mvaI                 mvaI                            sau3AI
                                                       ecoRII         ecoRII                    mboI/ndeII[dam-]
                                                       dsaV   tfiI    dsaV    pleI              dpnI[dam+]
                                                       bstNI     nlaIII     bstNI     ddeI                  dpnII[dam-]
             accI  nlaIII            mnlI              apyI[dcm+]   hinfI   apyI[dcm+]    hinfI    maeIII  alwI[dam-]
1101 CAAGTAAAGT AGACATGGTT TGGATAGTCG GAGGCAGTTC TGTTTACCAG GAAGCCATGA ATCAACCAGG CCACCTTAGA CTCTTTGTGA CAAGGATCAT
     GTTCATTTCA TCTGTACCAA ACCTATCAGC CTCCGTCAAG ACAAATGGTC CTTCGGTACT TAGTTGGTCC GGTGGAATCT GAGAAACACT GTTCCTAGTA

                                                                                          mnlI
                                                                                     hinlI/acyI          scrFI
                                                                                     ahaII/bsaHI         mvaI
                                                                                  scrFI          ecoRII
                                                                                  mvaI    ecoNI          dsaV
                                                                                  ecoRII      sau96I
                                                                                  dsaV        avaII
                         maeII                                                    bstNI   bslI     asuI    mnlI
                       aflIII                                                     apyI[dcm+]       mnlI  bstNI
         apoI       maeIII                                       mnlI          bsaJI hgaI     ddeI   apyI[dcm+]
1201 GCAGGAATTT GAAAGTGACA CGTTTTTCCC AGAAATTGAT TTGGGGAAAT ATAAACCTCT CCCAGAATAC CCAGGCGTCC TCTCTGAGGT CCAGGAGGAA
     CGTCCTTAAA CTTTCACTGT GCAAAAAGGG TCTTTAACTA AACCCCTTTA TATTTGGAGA GGGTCTTATG GGTCCGCAGG AGAGACTCCA GGTCCTCCTT

                                                                                                   ppu10I
                                                             sfaNI                                 nsiI/avaIII
         sfaNI                 accI     mboII            mboII                         mnlI     aluI
1301 AAAGGCATCA AGTATAAGTT TGAAGTCTAC GAGAAGAAAG ACTAACAGGA AGATGCTTTC AAGTTCTCTG CTCCCCTCCT AAAGCTATGC ATTTTTATAA
     TTTCCGTAGT TCATATTCAA ACTTCAGATG CTCTTCTTTC TGATTGTCCT TCTACGAAAG TTCAAGAGAC GAGGGGAGGA TTTCGATACG TAAAAATATT

                                                         fnu4HI
        nlaIII                                           aciI
       styI                                             thaI
       ncoI                                             fnuDII/mvnI tru9I
       dsaI                           styI              bstUI       mseI                                  maeIII
       bsaJI                          bsaJI             bsh1236I   aseI/asnI/vspI                        hphI
1401 GACCATGGGA CTTTTGCTGG CTTTAGACCC CCTTGGCTTC GTTAGAACGC GGCTACAATT AATACATAAC CTTATGTATC ATACACATAG ATTTAGGTGA
     CTGGTACCCT GAAAACGACC GAAATCTGGG GGAACCGAAG CAATCTTGCG CCGATGTTAA TTATGTATTG GAATACATAG TATGTGTATC TAAATCCACT
```

FIG. 6C

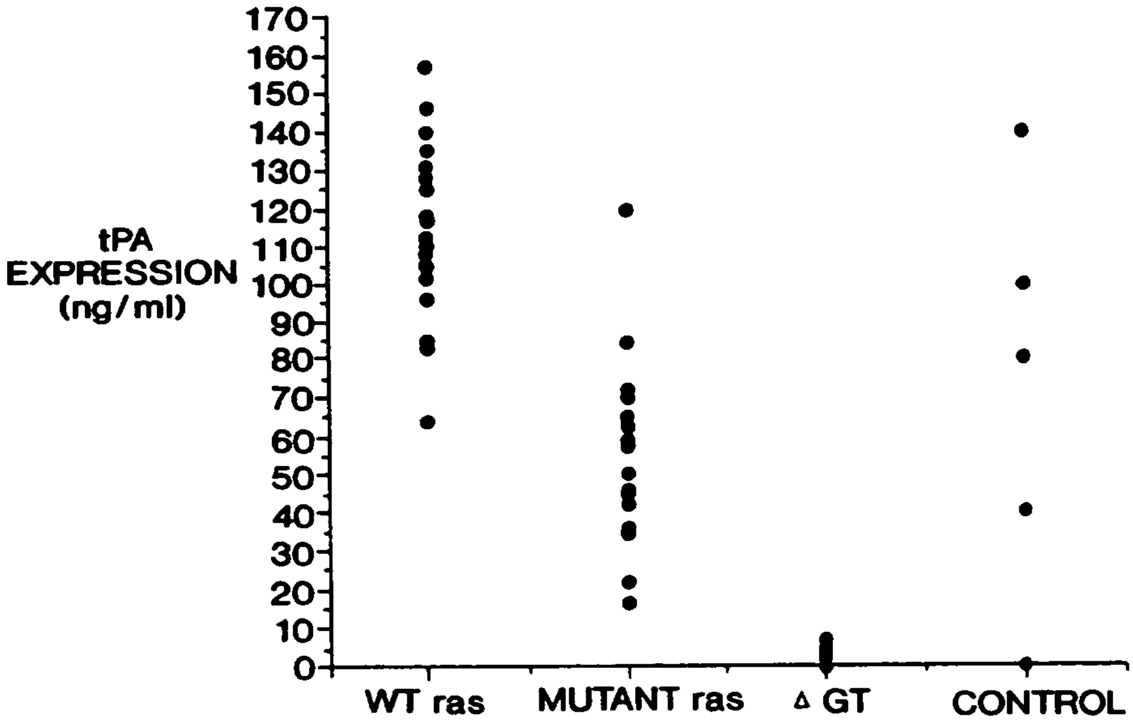
```
                tfiI
            aciI
           thaI hinfI
           fnuDII/mvnI                                                  fnu4HI
           bstUI                               aciI                                 bbvI
                                            rsaI                                  nspBII           taqI
           bsh1236I                         csp6I   scfI           mnlI           aciI       nlaIII
 701 TTGGAACGCG GATTCCCCGT GCCAAGAGTG CTGTAAGTAC CGCCTATAGA GCGATAAGAG GATTTTATCC CCGCTGCCAT CATGGTTCGA CCATTGAACT
     AACCTTGCGC CTAAGGGGCA CGGTTCTCAC GACATTCATG GCGGATATCT CGCTATTCTC CTAAAATAGG GGCGACGGTA GTACCAAGCT GGTAACTTGA

                                                                                  thaI
                                                                                  fnuDII/mvnI
                                                                                  bstUI
                                                                                  bsh1236I
                                                                                mluI
                                                                     bsrBI      aflIII     rsaI
                      pflMI                        bsmAI             aciI     xmnI         csp6I
     sfaNI            bslI                         bsaI       mnlI   ddeI asp700      scaI
 801 GCATCGTCGC CGTGTCCCAA AATATGGGGA TTGGCAAGAA CGGAGACCTA CCCTGCCCTC CGCTCAGGAA CGCGTTCAAG TACTTCCAAA GAATGACCAC
     CGTAGCAGCG GCACAGGGTT TTATACCCCT AACCGTTCTT GCCTCTGGAT GGGACGGGAG GCGAGTCCTT GCGCAAGTTC ATGAAGGTTT CTTACTGGTG

                                                            scrFI
                                                            mvaI
                                                            ecoRII
                                                            dsaV
          eco57I                                            bstNI
         mboII                 tfiI                                                 tfiI      tru9I         tru9I
        earI/ksp632I           hinfI                        apyI[dcm+]          mboII  taqI   mseI          mseI
       mnlI                  alwNI    hphI                sexAI             ddeI    hinfI    ahaIII/draI    aseI/asnI/vspI
 901 AACCTCTTCA GTGGAAGGTA AACAGAATCT GGTGATTATG GGTAGGAAAA CCTGGTTCTC CATTCCTGAG AAGAATCGAC CTTTAAAGGA CAGAATTAAT
     TTGGAGAAGT CACCTTCCAT TTGTCTTAGA CCACTAATAC CCATCCTTTT GGACCAAGAG GTAAGGACTC TTCTTAGCTG GAAATTTCCT GTCTTAATTA

                                              aluI
                                             sstI
                                             sacI
                                             hgiJII
                                             hgiAI/aspHI
                                             ecl136II
                                             bsp1286
                                             bsiHKAI
                                             bmyI                                   tru9I              mspI
                                             banII                           sfaNI  mseI               hpaII
           ddeI                   bslI    mnlI                bstXI      fokI      aflII/bfrI        bsaWI
1001 ATAGTTCTCA GTAGAGAACT CAAAGAACCA CCACGAGGAG CTCATTTTCT TGCCAAAAGT TTGGATGATG CCTTAAGACT TATTGAACAA CCGGAATTGG
     TATCAAGAGT CATCTCTTGA GTTTCTTGGT GGTGCTCCTC GAGTAAAAGA ACGGTTTTCA AACCTACTAC GGAATTCTGA ATAACTTGTT GGCCTTAACC
```

FIG. 6B

```
                                                                                   maeII
                                                                                  hinlI/acyI              nlaIV
               rsaI                          pleI                                 ahaII/bsaHI             hgiCI
               csp6I                  aciI   hinfI                bsmAI           aatII                   banI
401 GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA
    CCAAAACCGT CATGTAGTTA CCCGCACCTA TCGCCAAACT GAGTGCCCCT AAAGGTTCAG AGGTGGGGTA ACTGCAGTTA CCCTCAAACA AAACCGTGGT

                                                                                                          aluI
                                                                                                         sstI
                                                                                                         sacI
                                                                                                         hgiJII
                                                                                                         hgiAI/aspHI
                                                                                                         ecl136II
                                                                                                         bsp1286
                                                                                                         bsiHKAI
                                                                             rsaI                        bmyI
                                maeIII    aciI       hgaI        aciI        csp6I     mnlI              banII
501 AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT
    TTTAGTTGCC CTGAAAGGTT TTACAGCATT GTTGAGGCGG GGTAACTGCG TTTACCCGCC ATCCGCACAT GCCACCCTCC AGATATATTC GTCTCGAGCA

                                                                                                haeIII/palI
                                                                                               mcrI
                                                                                               eagI/xmaIII/eclXI
                                                                                               eaeI
                                                                                               cfrI
                                                                                              fnu4HI
                                                                                              aciI
                                                                                             thaI
                             esp3I                                                           fnuDII/mvnI
                        scrFI                                            sau96I          sacII/sstII
                        mvaI bsmAI                                             avaII           nspBII
                        ecoRII                                           asuI            kspI  scrFI
                        dsaV                                            nlaIV            dsaI  nciI
                        bstNI  hinlI/acyI                                  scrFI          bglI bslI  mspI
                        apyI[dcm+]                                         nciI      sau3AI mnlI bstUI
                   sau3AI gsuI/bpmI                                        mspI      mboI/ndeII[dam-] hpaII
                   mboI/ndeII[dam-]                                mboII   hpaII     dpnI[dam+] bsaJI dsaV
                   dpnI[dam+]  hgaI  fokI                          bpuAI   dsaV      dpnII[dam-] bsh1236I
                   dpnII[dam-] ahaII/bsaHI                mnlI     bbsI    cauII     alwI[dam-] aciI  cauII
601 TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA
    AATCACTTGG CAGTCTAGCG GACCTCTGCG GTAGGTGCGA CAAAACTGGA GGTATCTTCT GTGGCCCTGG CTAGGTCGGA GGCGCCGGCC CTTGCCACGT
```

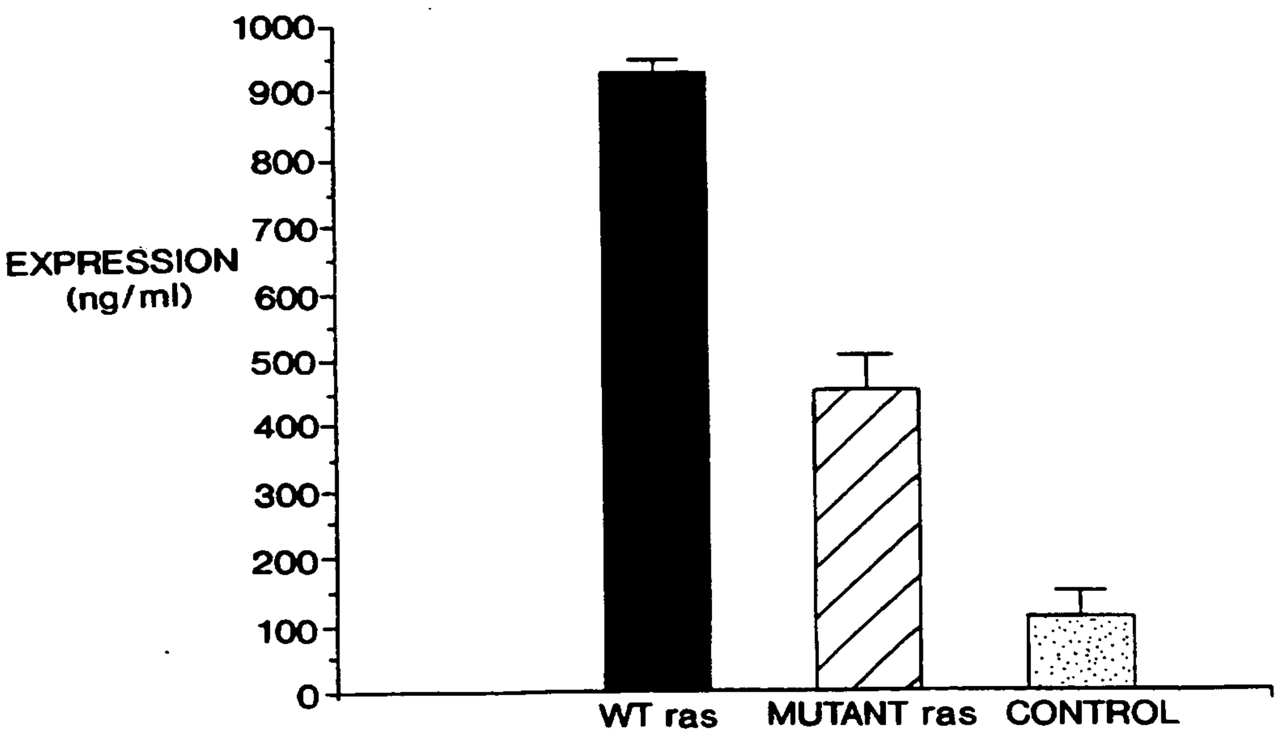
FIG. 6A

```
        aluI
       sstI
       sacI
       hgiJII
       hgiAI/aspHI
       ecl136II
       bsp1286                                                                                     thaI
       bsiHKAI                                                                                     fnuDII/mvnI
       bmyI                       rmaI    tru9I                                                    bstUI
       banII                      maeI    mseI                                                     bsh1236I
     taqI                        speI    aseI/asnI/vspI                        bslI            aciI maeIII
  1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC
    AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCAATAATTA TCATTAGTTA ATGCCCCAGT AATCAAGTAT CGGGTATATA CCTCAAGGCG CAATGTATTG

                      scrFI
                      mvaI
                      ecoRII
                      dsaV
                   aciI
                 bglI bstNI                                  maeII
                sau96I                                     hinlI/acyI
                haeIII/palI     aciI                       ahaII/bsaHI
                asuI  apyI[dcm+]               aciI        aatII          maeII           maeIII
101 TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA
    AATGCCATTT ACCGGGCGGA CCGACTGGCG GGTTGCTGGG GGCGGGTAAC TGCAGTTATT ACTGCATACA AGGGTATCAT TGCGGTTATC CCTGAAAGGT

       maeII                                                                                          maeII
      hinlI/acyI                                                                                     hinlI/acyI
      ahaII/bsaHI                                      rsaI                           rsaI           ahaII/bsaHI
      aatII                                  bglI         csp6I            ndeI          csp6I          aatII
201 TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT
    AACTGCAGTT ACCCACCTCA TAAATGCCAT TTGACGGGTG AACCGTCATG TAGTTCACAT AGTATACGGT TCATGCGGGG GATAACTGCA GTTACTGCCA

               scrFI
               mvaI
               ecoRII
           aciI                                                                                     nlaIII
         bglI  dsaV                                                                           styI
        sau96I bstNI            rsaI                                         maeII                 ncoI
        haeIII/palI             csp6I                               rsaI    snaBI                  dsaI hphI  aciI
        asuI   apyI[dcm+]    bsrI  nlaIII                           csp6I   bsaAI                  bsaJI   sfaNI
301 AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC
    TTTACCGGGC GGACCGTAAT ACGGGTCATG TACTGGAATA CCCTGAAAGG ATGAACCGTC ATGTAGATGC ATAATCAGTA GCGATAATGG TACCACTACG
```



NONAMPLIFIED

FIG. 5B



AMPLIFIED (200nM METHOTREXATE)

FIG. 5C

FIG. 10B

```
                                                                                   maeII
                                                                                  hinlI/acyI              nlaIV
               rsaI                          pleI                                 ahaII/bsaHI             hgiCI
               csp6I                 aciI    hinfI                bsmAI           aatII                   banI
401 GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA
    CCAAAACCGT CATGTAGTTA CCCGCACCTA TCGCCAAACT GAGTGCCCCT AAAGGTTCAG AGGTGGGGTA ACTGCAGTTA CCCTCAAACA AAACCGTGGT

                                                                                                          aluI
                                                                                                         sstI
                                                                                                         sacI
                                                                                                         hgiJII
                                                                                                         hgiAI/aspHI
                                                                                                         ecl136II
                                                                                                         bsp1286
                                                                                                         bsiHKAI
                                                                             rsaI                        bmyI
                                maeIII    aciI       hgaI        aciI        csp6I    mnlI               banII
501 AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT
    TTTAGTTGCC CTGAAAGGTT TTACAGCATT GTTGAGGCGG GGTAACTGCG TTTACCCGCC ATCCGCACAT GCCACCCTCC AGATATATTC GTCTCGAGCA

                                                                                                haeIII/palI
                                                                                               mcrI
                                                                                               eagI/xmaIII/eclXI
                                                                                               eaeI
                                                                                               cfrI
                                                                                              fnu4HI
                                                                                              aciI
                                                                                             thaI
                             esp3I                                                           fnuDII/mvnI
                        scrFI                                        sau96I           sacII/sstII
                        mvaI bsmAI                                          avaII            nspBII
                        ecoRII                                       asuI             kspI  scrFI
                        dsaV                                        nlaIV             dsaI  nciI
                        bstNI  hinlI/acyI                               scrFI          bglI bslI  mspI
                        apyI[dcm+]                                      nciI     sau3AI mnlI bstUI
                   sau3AI gsuI/bpmI                                     mspI     mboI/ndeII[dam-] hpaII
                   mboI/ndeII[dam-]                             mboII   hpaII    dpnI[dam+] bsaJI dsaV
                   dpnI[dam+]  hgaI  fokI                       bpuAI   dsaV     dpnII[dam-] bsh1236I
                   dpnII[dam-] ahaII/bsaHI             mnlI     bbsI    cauII    alwI[dam-] aciI  cauII
601 TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA
    AATCACTTGG CAGTCTAGCG GACCTCTGCG GTAGGTGCGA CAAAACTGGA GGTATCTTCT GTGGCCCTGG CTAGGTCGGA GGCGCCGGCC CTTGCCACGT
```

FIG. 10C

```
                tfiI                                                                             fnu4HI
            aciI                                                                                 aciI
           thaI hinfI                                                  bstXI                    thaI
           fnuDII/mvnI                         aciI                sau96I    styI               fnuDII/mvnI tru9I
           bstUI                     maeII rsaI            pleI scfI haeIII/palI                bstUI       mseI
           bsh1236I             maeIII    csp6I    scfI hinfI      asuI      bsaJI              bsh1236I   aseI/asnI/vspI
 701 TTGGAACGCG GATTCCCCGT GCCAAGAGTG ACGTAAGTAC CGCCTATAGA GTCTATAGGC CCACCCCCTT GGCTTCGTTA GAACGCGGCT ACAATTAATA
     AACCTTGCGC CTAAGGGGCA CGGTTCTCAC TGCATTCATG GCGGATATCT CAGATATCCG GGTGGGGGAA CCGAAGCAAT CTTGCGCCGA TGTTAATTAT

                                                                                                      sau96I
                                                                                                      avaII
                                                                                                      asuI
                                                                                                   scrFI
                                                                                                   mvaI
                                                                                                   ecoRII
                                                                                                   dsaV
                                                                                                   bstNI
                                                                                                   apyI[dcm+]
                                         maeIII
                                        hphI   scfI          fokI                              bslI bsaJI
 801 CATAACCTTA TGTATCATAC ACATACGATT TAGGTGACAC TATAGAATAA CATCCACTTT GCCTTTCTCT CCACAGGTGT CCACTCCCAG GTCCAACTGC
     GTATTGGAAT ACATAGTATG TGTATGCTAA ATCCACTGTG ATATCTTATT GTAGGTGAAA CGGAAAGAGA GGTGTCCACA GGTGAGGGTC CAGGTTGACG

                                                       hinlI/acyI
                                                       ahaII/bsaHI
                                                       aatII
                                                    thaI                       taqI[dam-]
                                                    fnuDII/mvnI              sau3AI                bsmAI
                  aluI  taqI                        bstUI                    mboI/ndeII[dam-]     esp3I
      mnlI       hindIII                           aciI maeII                dpnI[dam+]     tth111I/aspI
      bsaJI   ddeI     claI/bsp106             hphI bsh1236I    taqI         dpnII[dam-] taqI    hgaI          sfaNI
 901 ACCTCGGTTC TAAGCTTATC GATATGAAAA AGCCTGAACT CACCGCGACG TCTGTCGAGA AGTTTCTGAT CGAAAAGTTC GACAGCGTCT CCGACCTGAT
     TGGAGCCAAG ATTCGAATAG CTATACTTTT TCGGACTTGA GTGGCGCTGC AGACAGCTCT TCAAAGACTA GCTTTTCAAG CTGTCGCAGA GGCTGGACTA

                                                                                             hinPI
       aluI             tfiI                                                             fnu4HI
     fnu4HI             hinfI                taqI                                        bbvI
     bbvI      mnlI  mboII              aluI          mnlI                   aciI       aluI hhaI/cfoI
1001 GCAGCTCTCG GAGGGCGAAG AATCTCGTGC TTTCAGCTTC GATGTAGGAG GGCGTGGATA TGTCCTGCGG GTAAATAGCT GCGCCGATGG TTTCTACAAA
     CGTCGAGAGC CTCCCGCTTC TTAGAGCACG AAAGTCGAAG CTACATCCTC CCGCACCTAT ACAGGACGCC CATTTATCGA CGCGGCTACC AAAGATGTTT
```

FIG. 10D

```
                                                hinPI
                                                hhaI/cfoI    mspI
                                               thaI          hpaII
                                              aciI          mroI
                                          haeIII/palI       bspMII
                                         mcrI fnuDII/mvnI   bspEI
     sau3AI                              eagI/xmaIII/eclXI  bsaWI
     mboI/ndeII[dam-)                    eaeI bstUI      tfiI
     dpnI[dam+]                          cfrI bsh1236I   hinfI                       ecoRI
     dpnII[dam-]                     sfaNI  fnu4HI  bslI  accIII                     apoI                        sfaNI  aciI
1101 GATCGTTATG TTTATCGGCA CTTTGCATCG GCCGCGCTCC CGATTCCGGA AGTGCTTGAC ATTGGGGAAT TCAGCGAGAG CCTGACCTAT TGCATCTCCC
     CTAGCAATAC AAATAGCCGT GAAACGTAGC CGGCGCGAGG GCTAAGGCCT TCACGAACTG TAACCCCTTA AGTCGCTCTC GGACTGGATA ACGTAGAGGG

                                                                                                                   mcrI
                                                                                                                   eagI/xmaIII/eclXI
                                                                                                                   eaeI
                                                                                        styI                      fnu4HI
                                                                               thaI     ncoI           sau3AI  aciI
        hgiAI/aspHI                                                            fnuDII/mvnI             mboI/ndeII[dam-]
        bsp1286                                                      fnu4HI    bstUI    dsaI           dpnI[dam+]    sau3AI
        bsiHKAI                                                      bbvI  mcrI       haeIII/palI      dpnII[dam-]   mboI/ndeII[dam-]
        bmyI                                                       scfI    mspI bsh1236I bsaJI sfaNI    fnu4HI       dpnI[dam+]
        apaLI/snoI                                                 pstI    hpaII     mnlI nlaIII     pvuI/bspCI haeIII/palI
        alw44I/snoI     maeII                            nspBII                                                                
      bslI draIII  maeIII        bspMI                   aciI      bsgI  cfr10I aciI haeI    fokI  mcrI bbvI cfrI dpnII[dam-]
1201 GCCGTGCACA GGGTGTCACG TTGCAACACC TGCCTGAAAC CGAACTGCCC GCTGTTCTGC AGCCGGTCGC GGAGGCCATG GATGCGATCG CTGCGGCCGA
     CGGCACGTGT CCCACAGTGC AACGTTGTGG ACGGACTTTG GCTTGACGGG CGACAAGACG TCGGCCAGCG CCTCCGGTAC CTACGCTAGC GACGCCGGCT

                                  sau96I                                                      thaI       sau3AI
                                  avaII                                                       fnuDII/mvnI      nlaIII
                                  asuI                                                        bstUI      mboI/ndeII[dam-]
                          sau96I  rsrII/cspI                                                  bsh1236I   dpnI[dam+]
                 aciI     haeIII/palI  aciI    tfiI                                          hinPI       dpnII[dam-]
      ddeI      bsrBI     asuI     cpoI        hinfI           nlaIII                    ndeI hhaI/cfoI  alwI[dam-]
1301 TCTTAGCCAG ACGAGCGGGT TCGGCCCATT CGGACCGCAA GGAATCGGTC AATACACTAC ATGGCGTGAT TTCATATGCG CGATTGCTGA TCCCCATGTG
     AGAATCGGTC TGCTCGCCCA AGCCGGGTAA GCCTGGCGTT CCTTAGCCAG TTATGTGATG TACCGCACTA AAGTATACGC GCTAACGACT AGGGGTACAC

                                                   hinPI                                                           draIII
                                                   hhaI/cfoI                                                     nlaIV
                                                  thaI                                  bsaJI                    hgiCI
                                                  fnuDII/mvnI                         haeIII/palI                banI
                             tth111I/aspI         bstUI                 sfaNI        sau96I                     mspI
        bsrI              drdI             hgaI   bsh1236I    taqI    aluI           asuI mnlI        bslI      hpaII
1401 TATCACTGGC AAACTGTGAT GGACGACACC GTCAGTGCGT CCGTCGCGCA GGCTCTCGAT GAGCTGATGC TTTGGGCCGA GGACTGCCCC GAAGTCCGGC
     ATAGTGACCG TTTGACACTA CCTGCTGTGG CAGTCACGCA GGCAGCGCGT CCGAGAGCTA CTCGACTACG AAACCCGGCT CCTGACGGGG CTTCAGGCCG
```

FIG. 10E

```
                aciI
               thaI
               fnuDII/mvnI
         hgiAI/aspHI
         bsp1286
         bsiHKAI                                         aciI
         bmyI  bstUI                                    fnu4HI
         apaLI/snoI                                    haeIII/palI
         alw44I/snoI                                  eaeI          aciI          gsuI/bpmI              tfiI bslI
      mnlI       bsh1236I    nlaIV                    cfrI        nspBII        bsrI      mnlI           hinfI
1501 ACCTCGTGCA CGCGGATTTC GGCTCCAACA ATGTCCTGAC GGACAATGGC CGCATAACAG CGGTCATTGA CTGGAGCGAG GCGATGTTCG GGGATTCCCA
     TGGAGCACGT GCGCCTAAAG CCGAGGTTGT TACAGGACTG CCTGTTACCG GCGTATTGTC GCCAGTAACT GACCTCGCTC CGCTACAAGC CCCTAAGGGT

                                                                                                           fnu4HI
                                                                                                          thaI
                                                                                                          fnuDII/mvnI
                                                                                                          bstUI
                                                                                                          bsh1236I
                                                                                                         sacII/sstII
                                                                                                         nspBII
                                                                                                         kspI
                                                                                    mspI                 dsaI
                                                                                     hpaII               bsaJI
                                                                                    mroI                 aciI
                                                                                    bspMII              fnu4HI
                                                                                    bspEI              sau3AI aciI
                                   dsaI                                             bsaWI              mboI/ndeII[dam-]
                                 haeIII/palI                        rsaI     aciI    fokI              dpnI[dam+]
                          mboII  mnlI bsaJI              fnu4HI     csp6I  bsrBI   sfaNI    aluI       dpnII[dam-]
       mnlI            mboII   gsuI/bpmI                 bbvI   maeII   taqI    mnlI  accIII          alwI[dam-]
1601 ATACGAGGTC GCCAACATCT TCTTCTGGAG GCCGTGGTTG GCTTGTATGG AGCAGCAGAC GTACTTCGAG CGGAGGCATC CGGAGCTTGC AGGATCGCCG
     TATGCTCCAG CGGTTGTAGA AGAAGACCTC CGGCACCAAC CGAACATACC TCGTCGTCTG CATGAAGCTC GCCTCCGTAG GCCTCGAACG TCCTAGCGGC

         scrFI
         nciI
         mspI
         hpaII                                                                           aluI
         dsaV                                                                           fnu4HI                 hgaI
         cauII                                                                          bbvI      hinPI    taqI  drdI
      nlaIV               aciI                             aluI   nincII/hindII taqI  sfaNI      hhaI/cfoI  sfaNI
1701 CGGCTCCGGG CGTATATGCT CCGCATTGGT CTTGACCAAC TCTATCAGAG CTTGGTTGAC GGCAATTTCG ATGATGCAGC TTGGGCGCAG GGTCGATGCG
     GCCGAGGCCC GCATATACGA GGCGTAACCA GAACTGGTTG AGATAGTCTC GAACCAACTG CCGTTAAAGC TACTACGTCG AACCCGCGTC CCAGCTACGC
```

FIG. 10F

```
          nlaIV                                                haeIII/palI
         mspI                                                 mcrI
         hpaII  scrFI                                         eagI/xmaIII/eclXI
         bslI   nciI                                          eaeI
        mroI    mspI                                          cfrI
        bspMII  hpaII                                        fnu4HI
        bspEI[dam-]                                          aciI
        bsaWI   dsaV                                        thaI
        accIII[dam-]                                        fnuDII/mvnI
       sau3AI   cauII                                       bstUI
       mboI/ndeII[dam-]                                     bsh1236I    sau96I                rsaI
       dpnI[dam+]                                           hinPI       avaII                 csp6I
       dpnII[dam-]              rsaI                        hhaI/cfoI   asuI                 scaI
       alwI[dam-]               csp6I         aciI
1801 ACGCAATCGT CCGATCCGGA GCCGGGACTG TCGGGCGTAC ACAAATCGCC CGCAGAAGCG CGGCCGTCTG GACCGATGGC TGTGTAGAAG TACTCGCCGA
     TGCGTTAGCA GGCTAGGCCT CGGCCCTGAC AGCCCGCATG TGTTTAGCGG GCGTCTTCGC GCCGGCAGAC CTGGCTACCG ACACATCTTC ATGAGCGGCT

                                                                       scrFI
                                                                       nciI
                                                                       mspI
                                                                       hpaII
                                                                       dsaV
                                                                      xmaI/pspAI
                                                                      smaI
                                                                      scrFI
                                                                      nciI
                                                                      dsaV
                                                                      cauII
                                                                      bsaJI
                                                                      avaI
                                                                     bsaJI
                                                                   sau3AI                            sau96I
                                                                   mboI/ndeII[dam-]         aciI     haeIII/palI
                                                                   dpnI[dam+]              fnu4HI    asuI
                                                                   dpnII[dam-]             bglI  nlaIII
                                                                   alwI[dam-]             sfiI  styI
                                                                  nlaIV cauII            eaeI   ncoI
      hinlI/acyI                                         mcrI     bstYI/xhoII            cfrI   dsaI
      hgaI                    mnlI                      bslI      bamHI bsaJI ecoRI    taqI haeIII/palI
      ahaII/bsaHI           bsaJI                    sfaNI        alwI[dam-]  apoI    claI/bsp106 bsaJI
1901 TAGTGGAAAC CGACGCCCCA GCACTCGTCC GAGGGCAAAG GAATAGAGTA GATGCCGACC GAAGGATCCC CGGGGAATTC AATCGATGGC CGCCATGGCC
     ATCACCTTTG GCTGCGGGGT CGTGAGCAGG CTCCCGTTTC CTTATCTCAT CTACGGCTGG CTTCCTAGGG GCCCCTTAAG TTAGCTACCG GCGGTACCGG
```

FIG. 10G

```
                   aluI
                 fnu4HI
                 bbvI        maeIII              sfaNI   apoI                                  rmaI
                                                                                          bsmI maeI
2001 CAACTTGTTT ATTGCAGCTT ATAATGGTTA CAAATAAAGC AATAGCATCA CAAATTTCAC AAATAAAGCA TTTTTTTCAC TGCATTCTAG TTGTGGTTTG
     GTTGAACAAA TAACGTCGAA TATTACCAAT GTTTATTTCG TTATCGTAGT GTTTAAAGTG TTTATTTCGT AAAAAAAGTG ACGTAAGATC AACACCAAAC

                                       sau3AI
                                       mboI/ndeII[dam-]
                                       dpnI[dam+]
                                       dpnII[dam-]
                                      pvuI/bspCI
                                      mcrI
                                     taqI[dam-]  tru9I                haeIII/palI
                                    claI/bsp106[dam-]                haeI
                                   sau3AI        mseI        fnu4HI styI
                                   mboI/ndeII[dam-]          bbvI   ncoI
                                   dpnI[dam+] xmnI          hinPI   dsaI
                                   dpnII[dam-]  aseI/asnI/vspI      bsaJI
                            nlaIII  alwI[dam-]  asp700      hhaI/cfoI nlaIII        mnlI       mnlI
2101 TCCAAACTCA TCAATGTATC TTATCATGTC TGGATCGATC GGGAATTAAT TCGGCGCAGC ACCATGGCCT GAAATAACCT CTGAAAGAGG AACTTGGTTA
     AGGTTTGAGT AGTTACATAG AATAGTACAG ACCTAGCTAG CCCTTAATTA AGCCGCGTCG TGGTACCGGA CTTTATTGGA GACTTTCTCC TTGAACCAAT

      rsaI                                                           nlaIV
      csp6I                                                        scrFI                       sfaNI
     nlaIV                                                         mvaI                            ppu10I
     kpnI                                                          ecoRII                        nsiI/avaIII
     hgiCI                                                         dsaV                         nlaIII
     banI                      aluI                                bstNI                            sphI
     asp718    mnlI           pvuII                                apyI[dcm+]                       nspI
     acc65I  ddeI  aciI       nspBII                              bsaJI                             nspHI
2201 GGTACCTTCT GAGGCGGAAA GAACCAGCTG TGGAATGTGT GTCAGTTAGG GTGTGGAAAG TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC
     CCATGGAAGA CTCCGCCTTT CTTGGTCGAC ACCTTACACA CAGTCAATCC CACACCTTTC AGGGGTCCGA GGGGTCGTCC GTCTTCATAC GTTTCGTACG

                                          nlaIV
                  scrFI        scrFI
                  mvaI              mvaI                              ppu10I
                  ecoRII       ecoRII                        nsiI/avaIII
                  dsaV         dsaV                         nlaIII
                  bstNI             bstNI                          sphI
                  apyI[dcm+]        apyI[dcm+]                     nspI sfaNI
                 sexAI             bsaJI                           nspHI                           aciI
2301 ATCTCAATTA GTCAGCAACC AGGTGTGGAA AGTCCCCAGG CTCCCCAGCA GGCAGAAGTA TGCAAAGCAT GCATCTCAAT TAGTCAGCAA CCATAGTCCC
     TAGAGTTAAT CAGTCGTTGG TCCACACCTT TCAGGGGTCC GAGGGGTCGT CCGTCTTCAT ACGTTTCGTA CGTAGAGTTA ATCAGTCGTT GGTATCAGGG
```

FIG. 10H

```
                                                                                                     fnu4HI
                                                                                                     bglI
                                                              nlaIII                                sfiI
                                                             styI                                   haeIII/palI
                                                             ncoI                                 mnlI
                       aciI            bsrI             bslI dsaI                              haeIII/palI
               aciI fokI            aciI      aciI      aciI bsaJI                          mnlI bsaJI aciI
2401 GCCCCTAACT CCGCCCATCC CGCCCCTAAC TCCGCCCAGT TCCGCCCATT CTCCGCCCCA TGGCTGACTA ATTTTTTTTA TTTATGCAGA GGCCGAGGCC
     CGGGGATTGA GGCGGGTAGG GCGGGGATTG AGGCGGGTCA AGGCGGGTAA GAGGCGGGGT ACCGACTGAT TAAAAAAAAT AAATACGTCT CCGGCTCCGG

                                                                                       scrFI
                                                                                       nciI
                                                                                       mspI
                                                                                       hpaII
                                                                                       dsaV
                                                       styI                          haeIII/palI
                                                       bsaJI                       mcrI
                                                       blnI                        eagI/xmaIII/eclXI
                                                    haeIII/palI          rmaI      eaeI
         haeIII/palI                               stuI rmaI             maeI      cfrI
      mnlI     ddeI                   mnlI         haeI maeI            nheI      mspI cauII
      bsaJI mnlI  aluI              mnlI          mnlI avrII           aluI aluI  hpaII
2501 GCCTCGGCCT CTGAGCTATT CCAGAAGTAG TGAGGAGGCT TTTTTGGAGG CCTAGGCTTT TGCAAAAAGC TAGCTTATCC GGCCGGGAAC GGTGCATTGG
     CGGAGCCGGA GACTCGATAA GGTCTTCATC ACTCCTCCGA AAAAACCTCC GGATCCGAAA ACGTTTTTCG ATCGAATAGG CCGGCCCTTG CCACGTAACC

          tfiI
          hinfI                                                                      fnu4HI
       aciI                                                                          aciI
      thaI                                                  bstXI                  thaI
      fnuDII/mvnI                      aciI         scfI  sau96I   styI            fnuDII/mvnI tru9I
      bstUI               pleI       rsaI       pleI      haeIII/palI              bstUI       mseI
      bsh1236I            hinfI      csp6I scfI hinfI     asuI     bsaJI           bsh1236I  aseI/asnI/vspI
2601 AACGCGGATT CCCCGTGCCA AGAGTCAGGT AAGTACCGCC TATAGAGTCT ATAGGCCCAC CCCCTTGGCT TCGTTAGAAC GCGGCTACAA TTAATACATA
     TTGCGCCTAA GGGGCACGGT TCTCAGTCCA TTCATGGCGG ATATCTCAGA TATCCGGGTG GGGGAACCGA AGCAATCTTG CGCCGATGTT AATTATGTAT
```

FIG. 10I

```
                   sau3AI
                   mboI/ndeII[dam-]
                   dpnI[dam+]
                   dpnII[dam-]
                   alwI[dam-]
                 taqI[dam-]
               claI/bsp106[dam-]
              sau3AI
              mboI/ndeII[dam-]
              dpnI[dam+]
              dpnII[dam-]
             alwI[dam-]               fokI
2701 ACCTTTTGGA TCGATCCTAC TGACACTGAC ATCCACTTTT TCTTTTTCTC CACAGGTGTC
     TGGAAAACCT AGCTAGGATG ACTGTGACTG TAGGTGAAAA AGAAAAAGAG GTGTCCACAG

                                   sau96I
                                   avaII
                                   asuI
                                scrFI
                                mvaI                                rmaI
                                ecoRII              thaI    maeI
                                dsaV                fnuDII/mvnI
                                bstNI                     bstUI  nheI
                                apyI[dcm+]        mnlI    bsh1236I  aluI
       bslI  bsaJI                                bsaJI  nruI   aluI
     CACTCCCAGG TCCAACTGCA CCTCGGTTCG CGAAGCTAGC
     GTGAGGGTCC AGGTTGACGT GGAGCCAAGC GCTTCGATCG

                                nlaIII
                               styI
                               pflMI
                               ncoI
            sfaNI      ecoRI   dsaI
         fnu4HI taqI   apoI    bslI fokI                    rmaI
         bbvI claI/bsp106      bsaJI      nlaIII   fokI     maeI
2801 TTGGGCTGCA TCGATTGAAT TCCACCATGG GATGGTCATG TATCATCCTT TTTCTAGTAG
     AACCCGACGT AGCTAACTTA AGGTGGTACC CTACCAGTAC ATAGTAGGAA AAAGATCATC

                   rsaI               aluI
           gsuI/bpmI             pvuII tth111I/aspI
          bsrI  csp6I      ecoRV nspBII    bsrI
     CAACTGCAAC TGGAGTACAT TCAGATATCC AGCTGACCCA
     GTTGACGTTG ACCTCATGTA AGTCTATAGG TCGACTGGGT

           aluI
          sstI
          sacI
          hgiJII
          hgiAI/aspHI
          ecl136II
          bsp1286
          bsiHKAI
          bmyI                                    hphI
          banII          mnlI                    maeIII    bspMI
       avaI           aciI                      bstEII   hphI       bsrI
2901 GTCCCCGAGC TCCCTGTCCG CCTCTGTGGG CGATAGGGTC ACCATCACCT GCCGTGCCAG
     CAGGGGCTCG AGGGACAGGC GGAGACACCC GCTATCCCAG TGGTAGTGGA CGGCACGGTC

            taqI                                 bsrI
       hgaI              hphI   aluI   nlaIII
     TCAGAGCGTC GATTACGATG GTGATAGCTA CATGAACTGG
     AGTCTCGCAG CTAATGCTAC CACTATCGAT GTACTTGACC
```

FIG. 10J

```
                                                                                                     mspI
                                                                                                     hpaII
                                                                                                     bslI
                                                                gsuI/bpmI                           bsaWI
                                                               scrFI                        sau3AI
                                                               mvaI                              mboI/ndeII[dam-]
                                                     haeIII/palI                                 dpnI[dam+]
                  scrFI                      fnu4HI      ecoRII                      dpnII[dam-]
                  mvaI                             aciI        dsaV                        alwI[dam-]
                  ecoRII                    thaI mnlI   bstNI                             nlaIV
                  dsaV                      fnuDII/mvnI apyI[dcm+]  pleI                  bstYI/xhoII
                  bstNI    aluI                   bstUI     rsaI   pleI gsuI/bpmI                bamHI
                  apyI[dcm+]                      bsh1236I  csp6I  hinfI   hinfI                 alwI[dam-]
3001 TATCAACAGA AACCAGGAAA AGCTCCGAAA CTACTGATTT ACGCGGCCTC GTACCTGGAG TCTGGAGTCC CTTCTCGCTT CTCTGGATCC GGTTCTGGGA
     ATAGTTGTCT TTGGTCCTTT TCGAGGCTTT GATGACTAAA TGCGCCGGAG CATGGACCTC AGACCTCAGG GAAGAGCGAA GAGACCTAGG CCAAGACCCT

                                                                                      sau3AI                     styI
                                                                                      mboI/ndeII[dam-]           bsaJI
                                                                                      dpnI[dam+]             rsaI
                                                                                      dpnII[dam-]            csp6I
                                                                                      alwI[dam-]            nlaIV
                                   fnu4HI  mboII                                     nlaIV                  kpnI
                                   bbvI    bpuAI                                     bstYI/xhoII            hgiCI
                                 scfI      bbsI                                      bamHI rsaI             banI
                                 pstI   mspI                                      mnlI     csp6I            asp718
                                 bsgI   hpaII                               maeIII   alwI[dam-]             acc65I
3101 CGGATTTCAC TCTGACCATC AGCAGTCTGC AGCCGGAAGA CTTCGCAACT TATTACTGTC AGCAAAGTCA CGAGGATCCG TACACATTTG GACAGGGTAC
     GCCTAAAGTG AGACTGGTAG TCGTCAGACG TCGGCCTTCT GAAGCGTTGA ATAATGACAG TCGTTTCAGT GCTCCTAGGC ATGTGTAAAC CTGTCCCATG

              sau3AI
              mboI/ndeII[dam-]              mboII
              dpnI[dam+]        fnu4HI     bpuAI       aciI
              dpnII[dam-]       bbvI       bbsI    mboII                                           mnlI
3201 CAAGGTGGAG ATCAAACGAA CTGTGGCTGC ACCATCTGTC TTCATCTTCC CGCCATCTGA TGAGCAGTTG AAATCTGGAA CTGCCTCTGT TGTGTGCCTG
     GTTCCACCTC TAGTTTGCTT GACACCGACG TGGTAGACAG AAGTAGAAGG GCGGTAGACT ACTCGTCAAC TTTAGACCTT GACGGAGACA ACACACGGAC

                                                                                   scrFI
                                                                                   mvaI
                                                                                   ecoRII
                                                                                   dsaV
                              haeIII/palI                                          bstNI
       xmnI                  haeI     rsaI                     mnlI                apyI[dcm+]
       asp700               mnlI      csp6I                    bslI        maeIII bsaJI       maeIII
3301 CTGAATAACT TCTATCCCAG AGAGGCCAAA GTACAGTGGA AGGTGGATAA CGCCCTCCAA TCGGGTAACT CCCAGGAGAG TGTCACAGAG CAGGACAGCA
     GACTTATTGA AGATAGGGTC TCTCCGGTTT CATGTCACCT TCCACCTATT GCGGGAGGTT AGCCCATTGA GGGTCCTCTC ACAGTGTCTC GTCCTGTCGT
```

FIG. 10K

```
                                                                                                        sstI
                                                                                                        sacI
                                                                                                        hgiJII
                                                                                                        hgiAI/aspHI
                                                                                                        ecl136II
                                                                                                        bsp1286
                                                                                                        bsiHKAI
                                                                                                        bmyI
                                                                                                   haeIII/palI
                                                                                                  sau96I aluI
                                        ddeI                                                      asuI  banII
                    ddeI fnu4HI        celII/espI                                        hphI     eco0109I/draII
              scfI  mnlI  bbvI        bpu1102I                     accI                 maeIII    alwNI  ddeI
                                     hgaI
3401 AGGACAGCAC CTACAGCCTC AGCAGCACCC TGACGCTGAG CAAAGCAGAC TACGAGAAAC ACAAAGTCTA CGCCTGCGAA GTCACCCATC AGGGCCTGAG
     TCCTGTCGTG GATGTCGGAG TCGTCGTGGG ACTGCGACTC GTTTCGTCTG ATGCTCTTTG TGTTTCAGAT GCGGACGCTT CAGTGGGTAG TCCCGGACTC

                                                                 sau96I
                                                              nlaIII
                                                          aciI   haeIII/palI
                                                        fnu4HI   asuI
                                                        bglI stყI
                                              aluI     sfiI  ncoI
                                             hindIII  eaeI  dsaI                     aluI
                                           tru9I    cfrI  bsaJI                     fnu4HI
           maeIII     aluI                 mseI    taqI haeIII/palI                  bbvI          maeIII
3501 CTCGCCCGTC ACAAAGAGCT TCAACAGGGG AGAGTGTTAA GCTTCGATGG CCGCCATGGC CCAACTTGTT TATTGCAGCT TATAATGGTT ACAAATAAAG
     GAGCGGGCAG TGTTTCTCGA AGTTGTCCCC TCTCACAATT CGAAGCTACC GGCGGTACCG GGTTGAACAA ATAACGTCGA ATATTACCAA TGTTTATTTC

                                                                                                        sau3AI
                                                                                                        mboI/ndeII[dam-]
                                                                                                        dpnI[dam+]
                                                                                                        dpnII[dam-]
                                                                                                       pvuI/bspCI
                                                                                                       mcrI
                                                                                                      taqI[dam-]
                                                                                                     claI/bsp106[dam-]
                                                                                                    sau3AI
                                                                                                    mboI/ndeII[dam-]
                                                                                                    dpnI[dam+]
                                                       rmaI                                         dpnII[dam-]
         sfaNI   apoI                             bsmI maeI                               nlaIII   alwI[dam-]
3601 CAATAGCATC ACAAATTTCA CAAATAAAGC ATTTTTTTCA CTGCATTCTA GTTGTGGTTT GTCCAAACTC ATCAATGTAT CTTATCATGT CTGGATCGAT
     GTTATCGTAG TGTTTAAAGT GTTTATTTCG TAAAAAAAGT GACGTAAGAT CAACACCAAA CAGGTTTGAG TAGTTACATA GAATAGTACA GACCTAGCTA
```

FIG. 10L

```
                                                                         rsaI
                                 haeIII/palI                             csp6I
                                haeI                                    nlaIV
           tru9I      fnu4HI styI                                       kpnI
           mseI       bbvI   ncoI                                       hgiCI
          aseI/asnI/vspI     dsaI                                       banI                       aluI
        xmnI        hinPI    bsaJI                                      asp718     mnlI           pvuII
        asp700      hhaI/cfoI nlaIII          mnlI      mnlI            acc65I  ddeI  aciI        nspBII
3701 CGGGAATTAA TTCGGCGCAG CACCATGGCC TGAAATAACC TCTGAAAGAG GAACTTGGTT AGGTACCTTC TGAGGCGGAA AGAACCAGCT GTGGAATGTG
     GCCCTTAATT AAGCCGCGTC GTGGTACCGG ACTTTATTGG AGACTTTCTC CTTGAACCAA TCCATGGAAG ACTCCGCCTT TCTTGGTCGA CACCTTACAC

                                  nlaIV                                                                       nlaIV
                               scrFI                           sfaNI                 scrFI        scrFI
                               mvaI                                ppu10I                  mvaI               mvaI
                               ecoRII                        nsiI/avaIII             ecoRII       ecoRII
                               dsaV                         nlaIII                   dsaV         dsaV
                               bstNI                             sphI                      bstNI              bstNI
                               apyI[dcm+]                        nspI                      apyI[dcm+]         apyI[dcm+]
                              bsaJI                              nspHI                    sexAI              bsaJI
3801 TGTCAGTTAG GGTGTGGAAA GTCCCCAGGC TCCCCAGCAG GCAGAAGTAT GCAAAGCATG CATCTCAATT AGTCAGCAAC CAGGTGTGGA AAGTCCCCAG
     ACAGTCAATC CCACACCTTT CAGGGGTCCG AGGGGTCGTC CGTCTTCATA CGTTTCGTAC GTAGAGTTAA TCAGTCGTTG GTCCACACCT TTCAGGGGTC

                                    ppu10I
                                    nsiI/avaIII
                                   nlaIII
                                  sphI
                                  nspI sfaNI                                                                  bsrI
                                  nspHI                               aciI          aciI fokI aciI        aciI
3901 GCTCCCCAGC AGGCAGAAGT ATGCAAAGCA TGCATCTCAA TTAGTCAGCA ACCATAGTCC CGCCCCTAAC TCCGCCCATC CCGCCCCTAA CTCCGCCCAG
     CGAGGGGTCG TCCGTCTTCA TACGTTTCGT ACGTAGAGTT AATCAGTCGT TGGTATCAGG GCGGGGATTG AGGCGGGTAG GGCGGGGATT GAGGCGGGTC

                                                                    fnu4HI
                                                                    bglI
                         nlaIII                                    sfiI
                        styI                                       haeIII/palI
                        ncoI                                     mnlI    mnlI     ddeI
                   bslI dsaI                                 haeIII/palI bsaJI mnlI  aluI                 mnlI
       aciI        aciI bsaJI                             mnlI bsaJI aciI    haeIII/palI                mnlI
4001 TTCCGCCCAT TCTCCGCCCC ATGGCTGACT AATTTTTTTT ATTTATGCAG AGGCCGAGGC CGCCTCGGCC TCTGAGCTAT TCCAGAAGTA GTGAGGAGGC
     AAGGCGGGTA AGAGGCGGGG TACCGACTGA TTAAAAAAAA TAAATACGTC TCCGGCTCCG GCGGAGCCGG AGACTCGATA AGGTCTTCAT CACTCCTCCG
```

FIG. 10M

```
                 styI                                                                          scrFI
                 bsaJI                                                                         mvaI
                 blnI                                                                          ecoRII
              haeIII/palI                tru9I            haeIII/palI                          dsaV
             stuI rmaI                   mseI            eaeI                                  bstNI
             haeI maeI                  hpaI aluI        cfrI              maeIII          apyI[dcm+]       tru9I
            mnlI avrII             aluI hincII/hindII  bsrI               maeII    bsrI       bsaJI  maeIII     mseI
4101 TTTTTTGGAG GCCTAGGCTT TTGCAAAAAG CTGTTAACAG CTTGGCACTG GCCGTCGTTT TACAACGTCG TGACTGGGAA AACCCTGGCG TTACCCAACT
     AAAAAACCTC CGGATCCGAA AACGTTTTTC GACAATTGTC GAACCGTGAC CGGCAGCAAA ATGTTGCAGC ACTGACCCTT TTGGGACCGC AATGGGTTGA

                                                                       sau3AI
                                                                       mboI/ndeII[dam-]
                                                             sau96I    dpnI[dam+]
                                                             haeIII/palI
                                       aluI               mnlI  aciI   dpnII[dam-]
                fnu4HI                pvuII            mboII asuI     pvuI/bspCI
                bbvI  fokI            nspBII           earI/ksp632I   mcrI                        bglI
4201 TAATCGCCTT GCAGCACATC CCCCCTTCGC CAGCTGGCGT AATAGCGAAG AGGCCCGCAC CGATCGCCCT TCCCAACAGT TGCGTAGCCT GAATGGCGAA
     ATTAGCGGAA CGTCGTGTAG GGGGGAAGCG GTCGACCGCA TTATCGCTTC TCCGGGCGTG GCTAGCGGGA AGGGTTGTCA ACGCATCGGA CTTACCGCTT

       hinPI
       hhaI/cfoI
      nlaIV                                                                             hinPI
      narI                                                                              hhaI/cfoI
      kasI                                                                             thaI
      hinlI/acyI                                                                       fnuDII/mvnI              hinPI
      hgiCI                                                                            bstUI           hinPI    hhaI/cfoI
      haeII      aciI                                                                  bsh1236I        hhaI/cfoI
      banI   sfaNI                                                                  rsaI      scfI fnu4HI   tru9I
      ahaII/bsaHI                  sfaNI    aciI          aciI  maeII               csp6I     bslI aciI     mseI
4301 TGGCGCCTGA TGCGGTATTT TCTCCTTACG CATCTGTGCG GTATTTCACA CCGCATACGT CAAAGCAACC ATAGTACGCG CCCTGTAGCG GCGCATTAAG
     ACCGCGGACT ACGCCATAAA AGAGGAATGC GTAGACACGC CATAAAGTGT GGCGTATGCA GTTTCGTTGG TATCATGCGC GGGACATCGC CGCGTAATTC

                              fnu4HI
         aciI               hinPI
      fnu4HI                hhaI/cfoI                          hinPI
      aciI                 thaI                                hhaI/cfoI
     thaI                  fnuDII/mvnI                        haeII
     fnuDII/mvnI           bstUI                      hinPI rmaI
     bstUI                 bsh1236I     aciI          hhaI/cfoI   bsrBI
     bsh1236I    maeIII bbvI maeIII               haeII maeI   aciI             mboII                 maeII
4401 CGCGGCGGGT GTGGTGGTTA CGCGCAGCGT GACCGCTACA CTTGCCAGCG CCCTAGCGCC CGCTCCTTTC GCTTTCTTCC CTTCCTTTCT CGCCACGTTC
     GCGCCGCCCA CACCACCAAT GCGCGTCGCA CTGGCGATGT GAACGGTCGC GGGATCGCGG GCGAGGAAAG CGAAAGAAGG GAAGGAAAGA GCGGTGCAAG
```

FIG. 10N

```
                                    nlaIV
      mspI                         hgiJII                              mnlI
      hpaII                        bsp1286                          nlaIV
     naeI                          bmyI                             hgiCI
     cfr10I          aluI          banII        nlaIV               banI  taqI                  hphI
4501 GCCGGCTTTC CCCGTCAAGC TCTAAATCGG GGGCTCCCTT TAGGGTTCCG ATTTAGTGCT TTACGGCACC TCGACCCCAA AAAACTTGAT TTGGGTGATG
     CGGCCGAAAG GGGCAGTTCG AGATTTAGCC CCCGAGGGAA ATCCCAAGGC TAAATCACGA AATGCCGTGG AGCTGGGGTT TTTTGAACTA AACCCACTAC

         maeII   haeIII/palI
        draIII  sau96I                                     maeII  pleI        tru9I     pleI
        bsaAI   asuI                                      drdI    hinfI maeII mseI      hinfI        bsrI
4601 GTTCACGTAG TGGGCCATCG CCCTGATAGA CGGTTTTTCG CCCTTTGACG TTGGAGTCCA CGTTCTTTAA TAGTGGACTC TTGTTCCAAA CTGGAACAAC
     CAAGTGCATC ACCCGGTAGC GGGACTATCT GCCAAAAAGC GGGAAACTGC AACCTCAGGT GCAAGAAATT ATCACCTGAG AACAAGGTTT GACCTTGTTG

                                                                                                    thaI
                                                                                                    fnuDII/mvnI
                                                                                                    bstUI
                                                                     tru9I                          bsh1236I
            bslI                                                     mseI             tru9I      tru9I
           bslI  avaI                                     haeIII/palI        aluI     mseI  apoI mseI  apoI
4701 ACTCAACCCT ATCTCGGGCT ATTCTTTTGA TTTATAAGGG ATTTTGCCGA TTTCGGCCTA TTGGTTAAAA AATGAGCTGA TTTAACAAAA ATTTAACGCG
     TGAGTTGGGA TAGAGCCCGA TAAGAAAACT AAATATTCCC TAAAACGGCT AAAGCCGGAT AACCAATTTT TTACTCGACT AAATTGTTTT TAAATTGCGC

                                        hgiAI/aspHI
                                        bsp1286
                      maeII             bsiHKAI                                                       tth111I/aspI
                    tru9I               bmyI   ddeI                  aciI                            maeIII
         tru9I      mseI                apaLI/snoI rsaI             fnu4HI   tru9I                 maeII
         mseI   sspI  psp1406I          alw44I/snoI csp6I        sfaNI       mseI       aciI      bsaAI bsrI
4801 AATTTTAACA AAATATTAAC GTTTACAATT TTATGGTGCA CTCTCAGTAC AATCTGCTCT GATGCCGCAT AGTTAAGCCA ACTCCGCTAT CGCTACGTGA
     TTAAAATTGT TTTATAATTG CAAATGTTAA AATACCACGT GAGAGTCATG TTAGACGAGA CTACGGCGTA TCAATTCGGT TGAGGCGATA GCGATGCACT

                                                hinPI
                                                hhaI/cfoI             mspI
                                               thaI                   hpaII
                  hinPI                        fnuDII/mvnI            scrFI                    bsmAI
               fnu4HI                          bstUI                  nciI    aciI            esp3I
               bbvI                     nspBII bsh1236I               dsaV sfaNI             bslI
           nlaIII hhaI/cfoI   aciI      aciI  hgaI      drdI          cauII fokI          aluI maeIII
4901 CTGGGTCATG GCTGCGCCCC GACACCCGCC AACACCCGCT GACGCGCCCT GACGGGCTTG TCTGCTCCCG GCATCCGCTT ACAGACAAGC TGTGACCGTC
     GACCCAGTAC CGACGCGGGG CTGTGGGCGG TTGTGGGCGA CTGCGCGGGA CTGCCCGAAC AGACGAGGGC CGTAGGCGAA TGTCTGTTCG ACACTGGCAG
```

FIG. 100

```
                                                        thaI
                                                        fnuDII/mvnI
                                                        bstUI
                                                        bsh1236I
      scrFI                                            hinPI
      nciI                                             hhaI/cfoI                mnlI
      mspI      nspI                                  thaI                     haeIII/palI
      hpaII fnu4HI                                    fnuDII/mvnI         mboII      sau96I
      dsaV aluI nspHI                                 bstUI mnlI          bpuAI      asuI
      cauII bbvI nlaIII    mnlI   hphI      hphI      bsh1236I            bbsI      eco0109I/draII
5001 TCCGGGAGCT GCATGTGTCA GAGGTTTTCA CCGTCATCAC CGAAACGCGC GAGGCAGTAT TCTTGAAGAC GAAAGGGCCT CGTGATACGC CTATTTTTAT
     AGGCCCTCGA CGTACACAGT CTCCAAAAGT GGCAGTAGTG GCTTTGCGCG CTCCGTCATA AGAACTTCTG CTTTCCCGGA GCACTATGCG GATAAAAATA

                                                                            nlaIV
                                                                          aciI
                                                                         thaI
                                                                         fnuDII/mvnI
                                       maeII                             bstUI
                nlaIII                hinlI/acyI                         bsh1236I
        tru9I rcaI                    ahaII/bsaHI                       hinPI
        mseI  bspHI              ddeI aatII                             hhaI/cfoI
5101 AGGTTAATGT CATGATAATA ATGGTTTCTT AGACGTCAGG TGGCACTTTT CGGGGAAATG TGCGCGGAAC CCCTATTTGT TTATTTTTCT AAATACATTC
     TCCAATTACA GTACTATTAT TACCAAAGAA TCTGCAGTCC ACCGTGAAAA GCCCCTTTAC ACGCGCCTTG GGATAAACAA AATAAAAAGA TTTATGTAAG

                    rcaI
                    bspHI
                bsrBI   bsmAI                                          mboII
                aciI nlaIII                             sspI           earI/ksp632I
5201 AAATATGTAT CCGCTCATGA GACAATAACC CTGATAAATG CTTCAATAAT ATTGAAAAAG GAAGAGTATG AGTATTCAAC ATTTCCGTGT CGCCCTTATT
     TTTATACATA GGCGAGTACT CTGTTATTGG GACTATTTAC GAAGTTATTA TAACTTTTTC CTTCTCATAC TCATAAGTTG TAAAGGCACA GCGGGAATAA

                                                                                                hgiAI/aspHI
                                                                                                bsp1286
                                                                                     sau3AI     bsiHKAI
                                                                                     mboI/ndeII[dam-]
                                                                                     dpnI[dam+] bmyI
                                                                                     dpnII[dam-]
              fnu4HI                                                            eco57I          apaLI/snoI
              aciI                       hphI              hphI            sfaNI mboII[dam-]    alw44I/snoI  maeIII
5301 CCCTTTTTTG CGGCATTTTG CCTTCCTGTT TTTGCTCACC CAGAAACGCT GGTGAAAGTA AAAGATGCTG AAGATCAGTT GGGTGCACGA GTGGGTTACA
     GGGAAAAAAC GCCGTAAAAC GGAAGGACAA AAACGAGTGG GTCTTTGCGA CCACTTTCAT TTTCTACGAC TTCTAGTCAA CCCACGTGCT CACCCAATGT
```

FIG. 10P

```
            sau3AI
            mboI/ndeII[dam-]    sau3AI
            dpnI[dam+]          mboI/ndeII[dam-]
            dpnII[dam-]         dpnI[dam+]                  psp1406I        hgiAI/aspHI                  aciI
           bstYI/xhoII          dpnII[dam-]                 xmnI            bsp1286  tru9I               thaI
        bsrI         nspBII     alwI[dam-]                  asp700          bsiHKAI  mseI                fnuDII/mvnI
    taqI  alwI[dam-]  aciI     bstYI/xhoII              mboII maeII         bmyI   ahaIII/draI           bstUI
                                                                                                         bsh1236I
                                                                                                         hinPI
                                                                                                         hhaI/cfoI
5401 TCGAACTGGA TCTCAACAGC GGTAAGATCC TTGAGAGTTT TCGCCCCGAA GAACGTTTTC CAATGATGAG CACTTTTAAA GTTCTGCTAT GTGGCGCGGT
     AGCTTGACCT AGAGTTGTCG CCATTCTAGG AACTCTCAAA AGCGGGGCTT CTTGCAAAAG GTTACTACTC GTGAAAATTT CAAGACGATA CACCGCGCCA

                 scrFI
                 nciI
                 mspI
                 hpaII
                 dsaV
                 cauII
              hinlI/acyI                                                      rsaI
              hgaI                 aciI                                       csp6I  bsrI
              ahaII/bsaHI   bcgI   mcrI fnu4HI       ddeI                     scaI hphI   maeIII     sfaNI
5501 ATTATCCCGT GATGACGCCG GGCAAGAGCA ACTCGGTCGC CGCATACACT ATTCTCAGAA TGACTTGGTT GAGTACTCAC CAGTCACAGA AAAGCATCTT
     TAATAGGGCA CTACTGCGGC CCGTTCTCGT TGAGCCAGCG GCGTATGTGA TAAGAGTCTT ACTGAACCAA CTCATGAGTG GTCAGTGTCT TTTCGTAGAA

                                                                                                     sau96I
                                                                                                     avaII
                                                                                          sau3AI  asuI
                                                               haeIII/palI                mboI/ndeII[dam-]
                                                               eaeI                       dpnI[dam+]
                                                               cfrI                       dpnII[dam-]
                                  fnu4HI                       fnu4HI                     pvuI/bspCI
      fokI  nlaIII                bbvI       nlaIII            aciI                       mcrI  mnlI
5601 ACGGATGGCA TGACAGTAAG AGAATTATGC AGTGCTGCCA TAACCATGAG TGATAACACT GCGGCCAACT TACTTCTGAC AACGATCGGA GGACCGAAGG
     TGCCTACCGT ACTGTCATTC TCTTAATACG TCACGACGGT ATTGGTACTC ACTATTGTGA CGCCGGTTGA ATGAAGACTG TTGCTAGCCT CCTGGCTTCC

                                   nlaIII
                                sau3AI maeIII                         mspI
                                mboI/ndeII[dam-]     sau3AI   nlaIV
                                dpnI[dam+]           mboI/ndeII[dam-]
                               alwI[dam-]            dpnI[dam+]  hpaII
     aluI  aciI             nlaIII dpnII[dam-]       dpnII[dam-] bsaWI aluI                            maeIII
5701 AGCTAACCGC TTTTTTGCAC AACATGGGGG ATCATGTAAC TCGCCTTGAT CGTTGGGAAC CGGAGCTGAA TGAAGCCATA CCAAACGACG AGCGTGACAC
     TCGATTGGCG AAAAAACGTG TTGTACCCCC TAGTACATTG AGCGGAACTA GCAACCCTTG GCCTCGACTT ACTTCGGTAT GGTTTGCTGC TCGCACTGTG
```

FIG. 10Q

```
                                                                                 mspI
                                      hinPI                                      hpaII
                                    mstI                                        scrFI
                                    aviII/fspI       bsrI                  aluI nciI        tru9I      fokI
                fnu4HI          maeII hhaI/cfoI   tru9I                  rmaI   dsaV        mseI    bsrI        aciI
        sfaNI   bbvI           psp1406I           mseI                   maeI   cauII      aseI/asnI/vspI    mnlI
5801 CACGATGCCA GCAGCAATGG CAACAACGTT GCGCAAACTA TTAACTGGCG AACTACTTAC TCTAGCTTCC CGGCAACAAT TAATAGACTG GATGGAGGCG
     GTGCTACGGT CGTCGTTACC GTTGTTGCAA CGCGTTTGAT AATTGACCGC TTGATGAATG AGATCGAAGG GCCGTTGTTA ATTATCTGAC CTACCTCCGC

                                                                                                       aciI
                                      bglI                                       mspI             thaI
                                    sau96I                                       hpaII            fnuDII/mvnI
                  sau96I            haeIII/palI                                cfr10I             bstUI
                  avaII       hinPI asuI     mspI                           nlaIV  hphI       bsmAI            fnu4HI
                  asuI        hhaI/cfoI      hpaII                        gsuI/bpmI          bsaI bsh1236I     bbvI
5901 GATAAAGTTG CAGGACCACT TCTGCGCTCG GCCCTTCCGG CTGGCTGGTT TATTGCTGAT AAATCTGGAG CCGGTGAGCG TGGGTCTCGC GGTATCATTG
     CTATTTCAAC GTCCTGGTGA AGACGCGAGC CGGGAAGGCC GACCGACCAA ATAACGACTA TTTAGACCTC GGCCACTCGC ACCCAGAGCG CCATAGTAAC

                                                                                                           ddeI
             sau96I                                                                                   sau3AI
             asuI                                                pleI                                 mboI/ndeII[dam-]
            nlaIV                                                hinfI                                dpnI[dam+]
         bsrI haeIII/palI    mnlI                         eam1105I                 fokI               dpnII[dam-]
6001 CAGCACTGGG GCCAGATGGT AAGCCCTCCC GTATCGTAGT TATCTACACG ACGGGGAGTC AGGCAACTAT GGATGAACGA AATAGACAGA TCGCTGAGAT
     GTCGTGACCC CGGTCTACCA TTCGGGAGGG CATAGCATCA ATAGATGTGC TGCCCCTCAG TCCGTTGATA CCTACTTGCT TTATCTGTCT AGCGACTCTA

                                                                                                                 bphI
                                                                                                              rmaI
                                                                                                           sau3AI
                                                                                                           mboI/ndeII[dam-]
                                                                                                           dpnI[dam+]
          mnlI                                                                                     tru9I   dpnII[dam-]
      nlaIV                                                                        tru9I          ahaIII/draI maeI
      hgiCI         tru9I                                                          mseI        tru9I      bstYI/xhoII
      banI          mseI      maeIII                                             ahaIII/draI   mseI mseI  alwI[dam-]
6101 AGGTGCCTCA CTGATTAAGC ATTGGTAACT GTCAGACCAA GTTTACTCAT ATATACTTTA GATTGATTTA AAACTTCATT TTTAATTTAA AAGGATCTAG
     TCCACGGAGT GACTAATTCG TAACCATTGA CAGTCTGGTT CAAATGAGTA TATATGAAAT CTAACTAAAT TTTGAAGTAA AAATTAAATT TTCCTAGATC
```

FIG. 10R

```
                                                                                                  sau3AI
     sau3AI                                                                                       mboI/ndeII[dam-]
     mboI/ndeII[dam-]                                                                             dpnI[dam+]
     dpnI[dam+]                                                                                   dpnII[dam-]
     dpnII[dam-]                                                                                 bstYI/xhoII
     alwI[dam-]          nlaIII              maeII                                         sau3AI  alwI[dam-]
    bstYI/xhoII         rcaI             tru9I                                             mboI/ndeII[dam-]
   mboII[dam-]          bspHI            mseI                  ddeI hgaI                   dpnI[dam+] mboII[dam-]
                                                                                           dpnII[dam-]
6201 GTGAAGATCC TTTTTGATAA TCTCATGACC AAAATCCCTT AACGTGAGTT TTCGTTCCAC TGAGCGTCAG ACCCCGTAGA AAAGATCAAA GGATCTTCTT
     CACTTCTAGG AAAAACTATT AGAGTACTGG TTTTAGGGAA TTGCACTCAA AAGCAAGGTG ACTCGCAGTC TGGGGCATCT TTTCTAGTTT CCTAGAAGAA

                thaI                                                                  sau3AI
   sau3AI       fnuDII/mvnI                                                           mboI/ndeII[dam-]
   mboI/ndeII[dam-]                                                                   dpnI[dam+]
   dpnI[dam+]   bstUI                                                                 dpnII[dam-]
   dpnII[dam-]  bsh1236I                                                             alwI[dam-]
   alwI[dam-]  hinPI       fnu4HI                                  aciI             mspI
  bstYI/xhoII  hhaI/cfoI   bbvI                            aciI  nspBII             hpaII      aluI
6301 GAGATCCTTT TTTTCTGCGC GTAATCTGCT GCTTGCAAAC AAAAAAACCA CCGCTACCAG CGGTGGTTTG TTTGCCGGAT CAAGAGCTAC CAACTCTTTT
     CTCTAGGAAA AAAAGACGCG CATTAGACGA CGAACGTTTG TTTTTTTGGT GGCGATGGTC GCCACCAAAC AAACGGCCTA GTTCTCGATG GTTGAGAAAA

          bsrI          hinPI                    rmaI              haeIII/palI
      maeIII   eco57I   hhaI/cfoI                maeI      bslI   haeI                   scfI     aciI
6401 TCCGAAGGTA ACTGGCTTCA GCAGAGCGCA GATACCAAAT ACTGTCCTTC TAGTGTAGCC GTAGTTAGGC CACCACTTCA AGAACTCTGT AGCACCGCCT
     AGGCTTCCAT TGACCGAAGT CGTCTCGCGT CTATGGTTTA TGACAGGAAG ATCACATCGG CATCAATCCG GTGGTGAAGT TCTTGAGACA TCGTGGCGGA

                                                                     scrFI
                                                                     nciI
                                            fnu4HI                   mspI                       mspI
                                   alwNI    bbvI                     hpaII                      hpaII
                                  bsrI   fnu4HI                      dsaV     pleI            bsaWI      hinPI
      mnlI                      maeIII   bbvI      bsrI              cauII    hinfI         maeIII       hhaI/cfoI
6501 ACATACCTCG CTCTGCTAAT CCTGTTACCA GTGGCTGCTG CCAGTGGCGA TAAGTCGTGT CTTACCGGGT TGGACTCAAG ACGATAGTTA CCGGATAAGG
     TGTATGGAGC GAGACGATTA GGACAATGGT CACCGACGAC GGTCACCGCT ATTCAGCACA GAATGGCCCA ACCTGAGTTC TGCTATCAAT GGCCTATTCC

                                   hgiAI/aspHI
      mcrI                         bsp1286
     aciI                          bsiHKAI
    nspBII                         bmyI                                                                  hinPI
   fnu4HI                          apaLI/snoI                                                            hhaI/cfoI
   bbvI                            alw44I/snoI   aluI                      ddeI       scfI              haeII
6601 CGCAGCGGTC GGGCTGAACG GGGGGTTCGT GCACACAGCC CAGCTTGGAG CGAACGACCT ACACCGAACT GAGATACCTA CAGCGTGAGC ATTGAGAAAG
     GCGTCGCCAG CCCGACTTGC CCCCCAAGCA CGTGTGTCGG GTCGAACCTC GCTTGCTGGA TGTGGCTTGA CTCTATGGAT GTCGCACTCG TAACTCTTTC
```

FIG. 10S

```
                                                                                               scrFI
                                                                                               mvaI          scrFI
                                                                                               ecoRII  mvaI
                                             mspI                                              dsaV    ecoRII
                                             hpaII                                             bstNI         dsaV
                                             bslI    fnu4HI                  hinPI  mnlI       bsaJI         bstNI
                                08  aciI        bsaWI    aciI                    hhaI/cfoI   aluI  apyI[dcm+]    apyI[dcm+]
6701 CGCCACGCTT CCCGAAGGGA GAAAGGCGGA CAGGTATCCG GTAAGCGGCA GGGTCGGAAC AGGAGAGCGC ACGAGGGAGC TTCCAGGGGG AAACGCCTGG
     GCGGTGCGAA GGGCTTCCCT CTTTCCGCCT GTCCATAGGC CATTCGCCGT CCCAGCCTTG TCCTCTCGCG TGCTCCCTCG AAGGTCCCCC TTTGCGGACC

                                                                                                                 fnu4HI
                                                                                                                 aciI
                                                                                                                thaI
                                                                                                                fnuDII/mvnI
                                                     taqI                               nlaIV                   bstUI
                                    mnlI  drdI    hgaI           sfaNI             aciI                         bsh1236I
6801 TATCTTTATA GTCCTGTCGG GTTTCGCCAC CTCTGACTTG AGCGTCGATT TTTGTGATGC TCGTCAGGGG GGCGGAGCCT ATGGAAAAAC GCCAGCAACG
     ATAGAAATAT CAGGACAGCC CAAAGCGGTG GAGACTGAAC TCGCAGCTAA AAACACTACG AGCAGTCCCC CCGCCTCGGA TACCTTTTTG CGGTCGTTGC

                         haeIII/palI
                      scrFI
                      mvaI
                      ecoRII
                      dsaV                    nlaIII
                      bstNI bslI                   nspI
        bslI          apyI[dcm+]      haeIII/palI  nspHI                       tfiI
      haeIII/palI nlaIV haeI        haeI           aflIII                      hinfI                    aciI
6901 CGGCCTTTTT ACGGTTCCTG GCCTTTTGCT GGCCTTTTGC TCACATGTTC TTTCCTGCGT TATCCCCTGA TTCTGTGGAT AACCGTATTA CCGCCTTTGA
     GCCGGAAAAA TGCCAAGGAC CGGAAAACGA CCGGAAAACG AGTGTACAAG AAAGGACGCA ATAGGGGACT AAGACACCTA TTGGCATAAT GGCGGAAACT
```

FIG. 10T

```
                                                                                                                thaI
                                                                                                                fnuDII/mvnI
                                                                                                                bstUI
                                                                                                                bsh1236I
                                                                                                               hinPI
                                                                                                               hhaI/cfoI
                                                                                                              thaI
                                                                                hinPI                         fnuDII/mvnI
                           fnu4HI             fnu4HI                       sapI hhaI/cfoI                     bstUI
                           bbvI               bbvI  pleI                   mboII                              bsh1236I
                 bsrBI  aciI                hinPI   hinfI                  earI/ksp632I              mnlI    bslI
        aluI     aciI  fnu4HI      mcrI     hhaI/cfoI           mnlI   aciI   haeII              aciI       aciI
7001 GTGAGCTGAT ACCGCTCGCC GCAGCCGAAC GACCGAGCGC AGCGAGTCAG TGAGCGAGGA AGCGGAAGAG CGCCCAATAC GCAAACCGCC TCTCCCCGCG
     CACTCGACTA TGGCGAGCGG CGTCGGCTTG CTGGCTCGCG TCGCTCAGTC ACTCGCTCCT TCGCCTTCTC GCGGGTTATG CGTTTGGCGG AGAGGGGCGC

                    tru9I   aluI
         haeIII/palI       pvuII                                                     tru9I                      nlaIV
        eaeI tfiI   mseI   nspBII                                        hinPI       mseI      maeIII           hgiCI
        cfrI hinfI aseI/asnI/vspI                   bsrI     aciI        hhaI/cfoI  aseI/asnI/vspI mnlI         banI
7101 CGTTGGCCGA TTCATTAATC CAGCTGGCAC GACAGGTTTC CCGACTGGAA AGCGGGCAGT GAGCGCAACG CAATTAATGT GAGTTACCTC ACTCATTAGG
     GCAACCGGCT AAGTAATTAG GTCGACCGTG CTGTCCAAAG GGCTGACCTT TCGCCCGTCA CTCGCGTTGC GTTAATTACA CTCAATGGAG TGAGTAATCC

         scrFI
         mvaI
         ecoRII
         dsaV
         bstNI
         apyI[dcm+]              mspI                           aciI                                            xmnI
        bsaJI                    hpaII                        bsrBI                           aluI    nlaIII    asp700
7201 CACCCCAGGC TTTACACTTT ATGCTTCCGG CTCGTATGTT GTGTGGAATT GTGAGCGGAT AACAATTTCA CACAGGAAAC AGCTATGACC ATGATTACGA
     GTGGGGTCCG AAATGTGAAA TACGAAGGCC GAGCATACAA CACACCTTAA CACTCGCCTA TTGTTAAAGT GTGTCCTTTG TCGATACTGG TACTAATGCT

      tru9I
      mseI
     aseI/asnI/vspI
7301 ATTAA
     TAATT

>length: 7305
```

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/09576

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/64 C12N15/67 C12N15/85 C12N9/72 C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DNA CLONING, VOLUME III, EDITED BY D.M. GLOVER, 1987 IRL PRESS, OXFORD, GB;, pages 189-212, A.M.C. BROWN AND M.R.D. SCOTT 'Retroviral vectors'	1-3,7,8
Y	see page 192, line 7 - page 196, line 5; figures 2,3 --- -/--	5,6, 9-12, 16-21

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

2

Date of the actual completion of the international search	Date of mailing of the international search report
23 November 1995	08.12.95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Hornig, H

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	pZIP-Neo SV(B)1 see figure 1	5,6, 9-12, 16-21
Y	MOL. CELL. BIOL., vol. 5, no. 3, March 1985 ASM WASHINGTON, DC,US, pages 431-437, A.D. MILLER ET AL. 'Generation of helper-free amphotrophic retroviruses that tranduce a dominant-acting, methotrexate-resistant dihydrofolate reductase gene' see page 432, right column, line 5 - page 436, right column, line 7; figure 1	5,6, 9-12, 16-21
Y	WO,A,94 05784 (US) 17 March 1994 see the whole document	5,6, 9-12, 16-21
Y	EP,A,0 215 548 (ZYMOGENETICS INC ;UNIV WASHINGTON (US)) 25 March 1987 see the whole document	5,6, 9-12, 16-21
A	WO,A,92 17566 (GENENTECH INC) 15 October 1992 cited in the application see the whole document	1-21
A	WO,A,90 12025 (UNIV LELAND STANFORD JUNIOR) 18 October 1990 cited in the application see the whole document	1-21
A	EP,A,0 260 148 (GENENTECH INC) 16 March 1988 see the whole document	1-21
A	EP,A,0 160 457 (GENENTECH INC) 6 November 1985 cited in the application see the whole document	1-21

-/--

2

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PROC. NATL.ACAD SCI., vol. 86, February 1989 NATL. ACAD SCI.,WASHINGTON,DC,US;, pages 1041-1045, M. VIVAUD ET AL. 'A 5' splice-region G-C mutation in exon 1 of the human beta-globin gene inhibits pre-mRNA splicing: A mechanism for beta+-thalassemia' see the whole document -----	1-4

2

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		DE-T-	3688900	09-12-93
		JP-A-	62111690	22-05-87
		US-A-	4959318	25-09-90
		US-A-	5073609	17-12-91
		US-A-	5302529	12-04-94
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EP-A-0260148	16-03-88	AU-B-	613316	01-08-91
		AU-B-	7831787	19-05-88
		DE-A-	3730599	07-07-88
		FR-A-	2603899	18-03-88
		GB-A,B	2197321	18-05-88
		US-A-	5024939	18-06-91
		JP-A-	63152986	25-06-88
EP-A-0160457	06-11-85	AU-B-	601358	13-09-90
		AU-B-	4134585	24-10-85
		AU-B-	5295890	30-08-90
		EP-A-	0385558	05-09-90
		HK-A-	8395	27-01-95
		JP-A-	60243023	03-12-85
		JP-A-	6040942	15-02-94
		NO-B-	174934	25-04-94
		SG-A-	3994	10-06-94
		US-A-	4965199	23-10-90